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Research Article

# Pesticidal residue analysis and phytochemical screening in leaves and roots of *Barleria prionitis* Linn.

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### ABSTRACT

In the present study emphasize on phytochemical screening in leaves and roots of *Barleria prionitis* Linn. The extract of plant materials total ash content, extractive value, water soluble ash, and loss on drying values were identified. Medicinal plant materials are liable to contain pesticide residues, which accumulate from agriculture practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. It is therefore recommended that every sample of medicinal plants used for such type of studies should be analyzed for the pesticidal residues. Therefore in the present study parts of the herb used for their antidiabetic potential were analyzed for organochlorine pesticides, which are persistent and remain in the food chain for longer periods. The samples of leaf and root of *Barleria prionitis* were ground coarsely and then preceded for the extraction procedure. The sample of extracts were analysed for residues of organochlorine pesticides by Gas Chromatography (GC). Analysis were carried out on a Shimadzu Model 2010 Gas Chromatograph (GC) equipped with <sup>63</sup>Ni electron capture detector (ECD) and a capillary column HP ultra 2. The instrument was supported by Lab Solution software. The pesticide residues detected in them in the GC were within the limits. For phytochemical screening, the ethanolic and hydroalcoholic extracts obtained were prepared and subjected to various qualitative tests in order to reveal the presence or absence of common phytopharmaceuticals by using standard tests.

**Keywords:** Phytochemical screening, Pesticidal residues, *Barleria prionitis* Gas Chromatography, Ash value, extractive value.

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## 1. INTRODUCTION

Medicinal plant materials are liable to contain pesticide residues, which accumulate from agriculture practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. It is therefore recommended that every sample of medicinal plants used for such type of studies should be analyzed for the pesticidal residues. Therefore in the present study parts of the herb used for their antidiabetic potential were analyzed for organochlorine pesticides, which are persistent and remain in the food chain for longer periods. *Barleria prionitis*, used in this study is found in hedges, waste places in tropical parts throughout India. It is also found in Srilanka, Burma, Tropical Asia,

tropical S. Africa (Bukrill 1985). The whole plant, leaves, barks and roots are all used generally.<sup>1,2</sup>

The whole plant extract of *Barleria prionitis* contains iridoid glycosides, barlerin, and verbascoside, which have shown potent activity against respiratory syncytial virus in vitro and may account for the plants use in treating fever and several other respiratory diseases in herbal medicine (Balick et al, 1998). Extracts of plants have also been shown to effectively suppress the fungi Trychophyton mentagrophytes in vitro (Panwar et al 1979).<sup>3</sup> A mouthwash made from root tissue is used to relieve toothache and treat bleeding gums (Burkill 1985). The leaves are used to promote healing of wounds and to relieve joints pains and toothache (Parrota 2001). The extract of plant, due to its antiseptic

properties is incorporated into herbal cosmetics and hair products to promote skin and scalp health (Prakurti 2002; Probiotics New Zealand 2002; Vaipani 2002). The extract of root has further been observed to have 100% antifertility activity in male rats (Gupta et al 2000). Similar studies were also carried by using methanolic extract of root and antifertility activity was reported (Verma et al 2005).<sup>4</sup> The Iridoid enriched fraction from the ethanol water extract of aerial parts of the plant was evaluated for its hepatoprotective activity in various acute and chronic animal model of hepatotoxicity and significant hepatoprotection was observed (Singh et al 2005).<sup>5</sup>

### 1.1 Phytochemical Profile of *Barleria prionitis*:

The therapeutic or medicinal activity of plants usually depends on the presence of what are known as “active principles”, and some understanding of them is necessary in any study of the actions and uses of plant and plant parts as drugs. Chemical examination of the glyceride content of the roots of *B prionitis* revealed the presence of 8 fatty acids. They are capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid and linoleic acid. The authors highlighted the presence of a new acetylated Luteolin 7-O beta- D- Glucoside from the roots of *B. Prionitis*, (Gupta et.al, 1984) Flavonoids present in *B. prionitis* showed anti microbial activity (Nagarjun et.al., 1986).<sup>6,7</sup>

Chemical investigation of *B Prionitis* showed the presence of 4 sterols and 2 fatty acids. Four free amino acids were obtained from the defatted water extract of stem (Baxi et.al. 1990) Presence of luteolin-7-O (2"-O-p-coumaroyl) -B-D- glycopyranoside has been reported in the roots of *Barleria prionitis*.<sup>8,9</sup> The leaves and stems showed the presence of five iridoid glucosides (Purushottam et al 1988. The flowers are reported to contain scutallerin-7-neohesperoside and scutellarein-7-rhamnosyl glucoside. 5,6b,- epoxy-7b- hydroxy-8b-methyl-1b-D-rhamnosydl iridiod, 4-carbomethoxy-7b,

8a- dihydroxy-8b-methyl-1b-D-glucopyranosidyl iridiod, 6-O acetyl shanzhiside methyl ester; b- sitosterol also occur in the plant (Harborne et al 1971; Purushottam et al 1988 )<sup>10</sup>

## 2. MATERIALS & METHOD

### 2.1 Determination of Total Ash Value

Two gms of accurately weighed air dried powder formulation was taken in a previously weighed clean and dry platinum crucible incinerated at 450 C until the ash became free from carbon which was confirmed by the white colour of the ash. The crucible containing the ash was kept in a desiccator and allowed to cool till a constant weight was obtained.<sup>11</sup> The percentage total ash with reference to air-dried sample was calculated (I.P.1996 Vol-II). The results are given in Table 2.1

### 2.2 Determination of Water Soluble Ash Value

The ash was boiled with 25 ml of water for 5 minutes and filtered through ashless filter paper.<sup>12</sup> The residue collected on the filter paper was washed with hot distilled water. The filter paper was allowed to dry and ignited for 15 minutes at 450<sup>0</sup> C. The weight of insoluble ash was determined and subtracted from the total ash taken to obtain the water soluble ash.<sup>13</sup> The percentage of water soluble ash was calculated with reference to air dried sample (I.P.1996 Vol-II). The results are given in Table 2.1

### 2.3 Determination of Acid Insoluble Ash Value

The ash was boiled with 20 ml of hydrochloric acid (2M) for 5 minutes and filtered through ashless filter paper was washed with hot distilled water. The filter paper was allowed to dry and ignited to dull redness for 15 minutes and cooled in a desiccator and weighed.<sup>14</sup> The percentage of acid insoluble ash was calculated with reference to air dried sample (I.P.1996 Vol-II). The results are given in Table 1

**Table 1: Ash values of leaf and root samples of *Barleria prionitis*.**

		Total ash value %w/w	Water soluble ash %w/w	Acid insoluble ash %w/w
1	Leaf	17.64	8.24	0.988
2	Root	39.3	15.43	2.99

### 2.4 Solvent Extractive Values

The extraction of any drug material with a solvent yields a solution of different compounds. The composition of this solution will depend upon the drug and the solvent used. The use of a solvent can be the means of providing preliminary information on the quality of a particular drug sample (Kokate 1991).<sup>15</sup>

#### 2.4.1 Determination of Water Soluble Extractive

Five gms sample of leaf and root of *Barleria prionitis* was accurately weighed and macerated with 100 ml of chloroform water in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours and allowed to stand for 18 hours. The mixture was filtered

and the filtrate evaporated to dryness in a evaporating dish and finally dried in an oven at 105<sup>0</sup> C and weighed. The percentage of water-soluble extractive was calculated with reference to air dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2.2.<sup>16,17</sup>

#### 2.4.2 Determination of Ethanol Soluble Extractive

Five gms sample of leaf and root of *Barleria prionitis* was accurately weighed and macerated with 100 ml of 90% ethanol in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours and allowed to stand for 18 hours. The mixture was filtered and the filtrate evaporated to dryness in a tared flat-bottomed shallow dish and dried further at 105<sup>0</sup> C and

weighed. The percentage of ethanol soluble extractive was calculated with reference to air-dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2.2.<sup>18,19</sup>

### 2.4.3. Determination of Ether Soluble Extractive

Accurately weighed, twenty five grams of sample of leaf and root of *Barleria prionitis* was packed in a extraction thimble of soxhlet apparatus and 100 ml. Petroleum

ether (40 to 60) was slowly added to the thimble chamber. The distillation flask was heated at 40<sup>0</sup> C and extraction continued for 3 hours. The extract was collected and evaporated to complete removal of petroleum ether. The semisolid mass so obtained was weighed. The percentage of ether soluble with reference to air dried sample was determined (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2 n<sup>20,21</sup>

**Table 2: Extractive values of leaf and root of *Barleria prionitis***

		Water soluble extractive %w/w	Ethanol sol. Extractive %w/w	Ether soluble extractive %w/w
1.	Leaf	35.85	16.31	7.65
2.	Root	14.039	2.07	0.93

### 2.5 Determination of Loss On Drying

The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical changes or microbial contamination. Loss on drying or heating to constant weight can be determined for material which do not contain compounds, which are volatile at the temperature of drying. Two gm of sample of leaf and root of *Barleria prionitis* was accurately weighed and transferred in a previously weighed weighing bottle.<sup>22</sup> The bottle was stoppered loosely and placed in an oven at 105<sup>0</sup> C for 30 minutes. After drying the bottle was cooled to room temperature in a desiccator and weighed till a constant

weight was obtained. The loss on drying was calculated with reference to air- dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are given in Table 3<sup>23</sup>

### 2.6 Determination of Foreign Organic Matter

Approximately 100gm of the sample of leaf and root of *Barleria prionitis* was taken and spread out in a thin layer on a glass plate. The samples were inspected thoroughly through a magnifying glass and the foreign organic matters present in the sample were removed (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in the Table 3<sup>24</sup>

**Table 3: Loss on drying and foreign organic matter of leaf and root *Barleria prionitis*.**

	Loss on Drying %w/w	Foreign organic Matter
Leaf	18.43	Nil
Root	10.43	Nil

### 3.1 Preparation of Sample of pesticide analysis:

The samples of leaf and root of *Barleria prionitis* were ground coarsely and then preceded for the extraction procedure.<sup>25</sup> 5gm of individual sample was taken in a conical flask and 25ml of solvent mixture of Hexane and Acetone (1:1) was added to it. The combined mixture was shaken vigorously for 2-3 minutes and the extract was filtered through a Whatmann filter paper no.1 to another conical flask. The contents of the flask were re-extracted with 25ml of solvent mixture and the filtrate was collected into flask number 2.<sup>26</sup> The filtrate was then transferred to a 250ml capacity separating funnel and 2% of NaCl solution was added to it for the removal of acetone. The aqueous layer was transferred to another separating funnel of same capacity containing 15ml of hexane.<sup>27</sup> The contents of the sep. funnel were shaken vigorously for 2minutes. After clear separation of two layers, the lower layer was discarded.<sup>28</sup> The upper hexane layer was then transferred to the first separatory funnel and the combined extract was washed twice with 100ml portion of distilled water. Again the heavy lower layer was discarded and the hexane layer was subjected for clean up through chromatography.<sup>29</sup>

### 3.2 Column Clean Up:

The extracts were subjected to column clean up to remove any interfering pigments in water, vegetation samples. For this purpose column were prepared as per American Public Health Association manual (1995). A glass column was plugged tightly with glass wool.<sup>30</sup> It was then packed tightly with a layer of 5gm anhydrous Na<sub>2</sub>SO<sub>4</sub> followed by 5gm of 15% deactivated alumina.<sup>31</sup> In case of presence of any colored pigment as in vegetable extract, an additional layer of 0.5gm of deactivated charcoal was added to the column. The column was eluted with 10ml of HPLC grade hexane.<sup>32</sup>

### 3.3 Detection and Quantification:

The sample of extracts were analyzed for residues of organochlorine pesticides by Gas Chromatography (GC).<sup>33</sup> Analysis were carried out on a Shimadzu Model 2010 Gas Chromatograph (GC) equipped with <sup>63</sup>NI electron capture detector (ECD) and a capillary column HP ultra 2 (US 4293415) 0.52×25×0.32. The instrument was supported by Lab Solution software for

the analysis of different organochlorine pesticides. The results are given in the Table 2.1<sup>34</sup>

### 3.4 Instruments Parameters:

Injection temp	-	210 <sup>0</sup> C
Injection mode	-	Splitless
Carrier gas	-	N <sub>2</sub>
Pressure	-	100Kpa,
Total flow	-	22.9ml/min,
Column flow	-	3.31ml/min,
Linear velocity	-	57.4cm/sec,
Purge flow	-	57.4cm/sec.

### 3.5 Stock Standards:

The stock standards (2000ppm) of 15-mixture pesticide compound was obtained from Hewlett Packard Company, Wilmington, Delaware, USA Stock standard of 100ppm were prepared by diluting standard mixture of HPLC grade iso-octane and toluene.<sup>35</sup> These stocks were stored under freezing conditions. Working standards of mixture was prepared from 100-ppm stock solution. 0.5-1 ppm of this 15-mixture pesticide compound was used for calibrating the Gas chromatograph for analyzing residues of any of these known pesticides in the samples analyzed.<sup>36</sup>

**Table 4: Retention time of pesticides in Leaf and Root sample**

Leaf sample			Root sample	
S. No	Substance	Retention time	Substance	Retention time
1.	a-HCH	7.575	a-HCH	7.300
2	b-HCH	8.115	b-HCH	8.205
3	g-HCH	8.372	g-HCH	8.367
4	d-HCH	8.819	d-HCH	9.007
5	d-HCH	9.014	Heptachlor	10.420
6	d-HCH	9.203	Aldrin	11.451
7	Heptachlor	10.416	Aldrin	11.607
8	Heptachlor	10.647	Hept.epoxide	13.030
9	Aldrin	11.451	Dieldrin	14.860
10	Aldrin	11.780	4,4''-DDE	16.161
11	Hept.epoxide	13.058	Endrin	16.347
12	Hept.epoxide	13.276	-	-
13	Dieldrin	14.865	-	-
14	4,4''-DDE	16.188	-	-
15	4,4''DDE	16.367	-	-
16	Endosulphan-II	17.037	-	-
17	Endosulfate	18.618	-	-

## 4. RESULTS AND DISCUSSION

**4.1 Preliminary Phytochemical Screening:** The ethanolic and hydroalcoholic extracts obtained were subjected to various qualitative tests in order to reveal the presence or absence of common phytopharmaceuticals by using standard tests. Results recorded in table. Only the chlorinated hydrocarbons and

related pesticides (eg aldrin, BHC, chlordane, dieldrin, DDT) and few organophosphorus pesticides (eg carbophenothion) retain a long residual action. Most other pesticides have very short residual actions. Therefore, when the length of the exposure to pesticides is unknown, the medicinal plant material should be tested for the presence, or the content determined of organically - bound chlorine.

**Table 5: Qualitative test of phytochemicals in the leaf and root sample of *Barleria prionitis***

1	Alkaloids	Mayer's reagent, Wagner's reagent, Dragendorff's reagent	Absent
2	Flavanoids	Ammonia test, Alkaline reagent test	Present
3	Tannins	Acetate test, Bromine water test	Present
4	Steroids	Salkowski test	Present
5	Protein	Biuret test, Ninhydrin test	Present
6	Gums and Resins	Ferric chloride test and HCl test	Absent
7	Terpenoids	With Chloroform and conc. Sulphuric acid	Present
8	Glycosides	Bontrager's test	Present
9	Saponins	Frothing test	Present

Phytochemical analysis showed the presence of flavanoids, alkaloids, tannins, sterols, protein,

glycosides, terpenoids and saponins. Limits of the pesticide residues unless otherwise indicated in the



monograph the drug should comply with the limits given in the 1996 addendum to the 1993 British Pharmacopoeia. The limit applying to pesticides that are not listed in the above said table and the presence of which is suspected for any reason must comply with the limits set by European Community Directives C/90/642, including their annexure and updates. Limits for pesticides which are not listed in the table nor in the EC Directives are calculated using the formula 1 given below.

## CONCLUSION

All the values were found in acceptance criteria viz. loss on drying, water soluble ash value, extractive value and phytochemical analysis showed the presence of flavanoids, alkaloids, tannins, sterols, protein,

glycosides, terpenoids and saponins. The phytochemical profile clearly indicated the numerous uses of *Barleria prionitis* by the folklore and it can prove beneficial for many diseases and specially diabetes. A maximum residue limit (MRL) for medicinal plant materials, including their preparations such as tinctures, extracts, oils, etc. may be defined in line with the limits of pesticide residues set by the FAO/WHO Codex Alimentarius at the lowest level acceptable for vegetable food products. Since medicinal plant materials are usually taken in much smaller quantities than other food products MRL can be calculated based on the maximum acceptable daily intake of pesticides for humans (ADI) and the maximum daily dose of the medicinal plant material (MDD).

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