EXPLORATION OF LEAVES, STEM AND ROOTS OF BRYOPHYLLUM PINNATUM FOR ITS PHARMACOLOGICAL AND PHYTOCHEMICAL PROFILE.

Harender Sharma¹, Avijit Mazumder², Bharti Yadav, Amit bind³

¹,²,³Department of pharmacology, Noida Institute of Engineering and Technology (Pharmacy Institute), avijitmazum@yahoo.com

ABSTRACT

Bryophyllum pinnatum (Lam.) Kurz (belongs to family Crassulaceae) was a perpetual growing plant that occur broadly in the stifling equatorial Africa, USA, Bharat, Australia and was used in folkloric medicine. Bryophyllum pinnatum (Lam) Pers. was endemic to Madagascar and Brazil both. As the numbers of diseases were increasing daily, there was a shift in focus to use herbal remedies as compared to the synthetic counterparts. This attracts the researcher towards the natural remedies. We have selected Bryophyllum pinnatum as our plant of study. Detailed informations pertaining to the phytochemical and pharmacological studies of the plant were gathered in this review were collected by using electronic search as PubMed, Research gate, Elsevier, Google scholar and Web science. Triterpenoids, glycosidal, flavonoidal, steroidal moieties along with alkaloids and bufadienolides were among some of the active compounds contained in this holy plant. The plant had a considerable high level of alkaloids with highest value in the leaf (2.08± 0.02 mg/100g). The stem extract had the highest value of saponins (0.81± 0.014 mg/100g) and the root extract had the highest level of sterols (0.49± 0.014 mg/100g). The plant had been found to possess antimicrobial activity, anti-inflammatory, antidiabetic, anticonvulsant, antihypertensive and anticancer activity, antitumour, anthelmentic, hepatoprotective, inflammatory, nephroprotective, antioxidant activity, anticonvulsant, neuropharmacological, antipyretic, haemostatic and wound healing properties. By observing the pharmacological and active constituents of the roots, stem and leaves various herbal formulations can be prepared to fight various disorders with fewer side effects.

Keywords: Bryophyllum Pinnatum, pharmacological activity, roots, stem, leaf.

I. INTRODUCTION

Bryophyllum pinnatum, brief (Crassulaceae) Synonym- Kalanchoe pinnata, patharchitta, maternity plant, Cathedral bell, parnabija. The leaves have a rooting vegetative bud on them (1). In the Bengal areas, the plant was popular as Patharkuchi and was a drug that was widely used to cure urinary stones (2). The plant can be found growing in hot, humid areas all over India, majorly in Bengal region. This was a fleshy annual herb and develops to a height of 1-1.5 meters and has a hollow four-angled stem that was commonly branched. The leaves were 10-20 cm long, opposite, decussate, and succulent. Lower leaves were simple, while upper leaves were 3-7 long-petioles and foliated. These were moist dark green with scallop edges and a red trim present. Bijeugate compound leaf blade, 10–30 cm long, with 3–5 leaflets. The blades of the leaflet were oblong to elliptic in shape, 6–8 x3–5 cm, and petiolules were 2–4 cm. Each notch on the margin bears a dormant bud capable of developing into a healthy plantlet (3). It has 5cm tall, purple-reddish, flowers that dangle in wide outspreading panicles; its fruits were membranous follicles covered in a papery corolla and calyx, with small ellipsoid seeds. The taxonomical classification was mentioned in Table.1 (4). The picture of Bryophyllum pinnatum plant was shown in Fig.1.
Table 1 - Taxonomical classification (4)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae – Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub kingdom</td>
<td>Tracheobionta – Vascular plants</td>
</tr>
<tr>
<td>Division</td>
<td>Spermatophyta – seed plants</td>
</tr>
<tr>
<td>Subdivision</td>
<td>Magnoliophyta – Flowering plants</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida – Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosidae Order : Rosales</td>
</tr>
<tr>
<td>Family</td>
<td>Crassulaceae – stonecrop</td>
</tr>
<tr>
<td>Genus</td>
<td>Bryophyllum</td>
</tr>
<tr>
<td>Species</td>
<td>Bryophyllum pinnatum</td>
</tr>
</tbody>
</table>

PHYSICOCHEMICAL PARAMETERS
The finding shows 4.19% water soluble ash, 1.69% acid insoluble ash, 5.60% alcohol soluble extractive value, 5.1% Total ash and 19.80%, water soluble extractive value (5).

ETHNOPHARMACOLOGY
It was used to cure and manage a variety of diseases including pink eye, haemorrhoids, burns, dermatitis, edema, constipation problems, convulsions, pneumonia, colds, menstrual problems, mild fever all over the world. It has anti-inflammatory, wound-healing, analgesic, and hemostatic properties. Hepatoprotective, laxative, diuretic and anti-psychotic properties were all claimed for root extract (6). In Torrid Zone where the plants occur naturally, for example Madagascar, *Bryophyllum pinnatum* has long been traditionally used for medicinal uses. The bitter leaves and stems were protective against diarrhoea, flatulence, and vomiting due to their astringent properties. *Bryophyllum pinnatum* plants have an unusual tendency to grow many new plantlets from the leaves. Thus implies a solid vegetative power and great vitality, according to anthroposophic principles. Because of its sedative effects, *B. pinnatum* was dubbed "herbal valium" because it was traditionally used to relieve inner restlessness and anxiety (7).

PHYTOCHEMICAL PROFILE
The plant fractions were tested for the presence of tannins, sugars (reducing), steroids, glycosides, resins, alkaloids, anthraquinones, flavonoids and flavonosides, and using normal phytochemical techniques. In several fractions of the plant, phytochemical research revealed the existence of tannins and reducing sugars. Only the n-hexane (soluble) containing fraction of *Bryophyllum pinnatum* contains anthraquinones (8). There were constituents like kaempferol, 3, 8-dimethoxy-4,5,7-trihydroxyflavone, astragalin, friedelin, epigallocatechin-3-o-syringate, rutin, luteolin and quercetin were all present in the leaves (1).

Other constituents were β-sitosterols, Bryophyllin B (Fig.2) having anti-tumour property, Bryophyllin A also called as Bryotoxin C, a bufadienolide1, 3, 5-orthoacetate having high cytotoxicity, and an insecticidal property bufadienolide (known as Bryophyllin C) were among the cardienolides and steroidal contents(9).
The leaves include bufadienolides such as Bryotoxin A (Fig.3), B, and C, which have antibacterial, antitumor, cancer-preventive, and insecticidal properties and were structurally and functionally identical to glycosides digitoxin and the other digoxin (10).

**Fig.2 Bryophyllin B**

**Fig.3 Bryotoxin A**

**PHARMACOLOGICAL ACTIVITY OF LEAVES**

**NEUROSEDATIVE**

Oral administration of *B. pinnatum* aqueous extract at doses ranging between 50- 200 mg/kg resulted in substantial depression of CNS effects. On pentobarbitone-induced hypnosis, it resulted in lengthen of the onset and sleep duration according to dose. Ten mice of different sexes were administered the various doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg of Bryophyllum* pinnatum* extract, chlorpromazine hydrochloride 4 mg/kg, half an hour after injection of the control group of mice with 10 ml/kg of extract solution. Each animal received sodium pentobarbitone (40 mg/kg). The time interval period between the loss and the regeneration of righting reflex was used to calculate the sleeping time by evasion and hole-board method were also reported (11). Shetty et.al had reported that bufadienolides were reported to possess CNS depressant activity (12).

**MUSCLE RELAXANT ACTIVITY**

The climbing method was used in which animals had earlier been taught for ascend a 60 cm chain hanging from a retorting stand clamp, which was 100 centimetre above ground level. Those mice that ascended that long chain in less than 10 seconds were selected for further experiment. Each animal was positioned with all four limbs grasping the chain end, and the duration it took for at the minimum two fore limbs to meet the long chain's top was recorded. This criteria was performed for 30 minutes after *Bryophyllum pinnatum* extract dosing range 50 mg/kg, 100 mg/kg, 200 mg/kg and diazepam(1mg/kg) intra muscular or saline solution (n=10) administration. The muscle relaxant behaviour was also tested using a chimney test, an inclined screen test, and a traction test. Shetty et.al had reported that major active constituent for the muscle relaxant property considered was glycosides and flavonoids.

**ANTICONVULSANT ACTIVITY**

In strychnine-induced convulsion model, saline was given to control group and *Bryophyllum pinnatum* extract (50 - 200 mg/kg) were assigned to mice of any sex having n=6 at random. 30 minutes after procedure, mice were given strychnine HCL (4.0 mg/kg, i.m route), and the initiation of tonic convulsion, as well as the count of mice exhibiting tonic type convulsion and impermeance, were reported. As a control, pentobarbitone sodium 40 mg/kg intraperitoneally was given (13).

**ANTI INFLAMMATORY ACTIVITY**

The Ethanolic extract of plant was screened to possess flavonoid, rutin and quercetin along with luteolin 7-O—D-glucoside moiety. As it inhibits the arachidonic acid cascade in both acute and chronic inflammatory disorders, it worked well as a local anti-inflammatory drug. The disparity in between weight of the inflamed and non-inflamed ears was used to determine edema. The rise in the weight of the ear after the inflammation
challenge was then calculated. Edema was measured with a digital micrometer before and after (basal ear thickness) activation of the inflammation response, the animals anaesthetized by (ketamine + xylazine) 80 mg/kg and15 mg/kg respectively, in the chronic model (multiple applications). Chibli et al. had reported that flavonoids block many inflammatory targets, particularly the arachidonic acid pathway, may be involved in anti-inflammatory effects (14).

ANTIMICROBIAL ACTIVITY
The antimicrobial property of hexane, ethanol, and methanolic extracted fractions, additionally isolated active compounds, was investigated. On a range of germs tested, the crude extracts showed antibacterial and antifungal activity, with the Minimum Inhibitory Concentration values ranging from 16 - 1024 g/ml. The ethanolic extract was found more active (MIC value = 16-128 g/ml) apart the methanolic extract whose MIC value = 32-512 g/ml or the hexane extract having MIC = 128-1024 g/ml, implying that active constituent in the ethanolic extract were more concentrated. In certain circumstances, the antimicrobial efficacy of the compound was comparable to that of the positive controls of nystatin and ciprofloxacin. Despite this, antibacterial efficacy of this compound (MIC = 1 g/ml) against Pseudomonas aeruginosa was greater than the positive control which was ciprofloxacin having MIC value = 2 g/ml. Tatsimo et al. reported that alpha-rhamnoisorobin is most active among Kaempferolrhamnosides had MIC values higher than the reference drug (15).

ANTI-CANCER
The presence of dose-dependent anti-tumor activity in extract of leaf in fraction of ethyl acetate and petroleum ether of 50:50 suggested that this medicinal plant could be used to treat cervical cancer. Ethyl acetate and petroleum ether extract showed IC50 at 91μg/ml. On cervical cancer cells, the anti-viral action of the extract with its fraction was demonstrated by down - regulation of API's special DNA binding activity when it was constitutively involved and repression of cJun expression and oncogenic c-Fos, as well as inhibition of HPV18 transcription (16).

Using the human cancer cell line HeLa, the anticancer properties of Bryophyllumpinnatum lectin (BL) were discovered using the SRB test. Graph pad prism software was used to analyse the results. The influence of plant lectin at 200g/ml concentration on HeLa (cervical cancer cell line) was successful after 48 hours, according to the anticancer properties of Bryophyllumpinnatum lectin (BL) as determined by the SRB assay. Flow cytometry was used to examine cell cycle and apoptosis using Propidium iodide and Annexin V FITC (Fluorescein isothiocyanate) with 200g/ml of Bryophyllumpinnatum lectin (17). Yamagishi Tet.al had showed bufadienolides are responsible for the cytotoxicity (18).

TOCOLYTIC
The key cause of perinatal death and many long term morbilities were prematurity that was birth earlier then 37 weeks of pregnancy and preterm labour (19).Tocolytics like adrenergic receptor agonists, calcium channel blockers and oxytocin antagonists were significant therapeutic factor and preterm uterine contractions were strongly linked to preterm delivery. The bufadienolide-containing fraction was the highly effective, with 1 g/mL lowers region under curve up to 40 %, while 150 g/mL of flavonoid filled fraction, flavonoid aglycon mixture (6.2 g/mL), and 10 g/mL of bryophyllumpinnatum juice of the leaf were expected to reach comparable inhibition. Santos et al. had reported bufadienolide highly effective for tocolytics activity (20).

ANTI HYPERTENSIVE
Normotensive wistar rats were given 18 percent NaCl (1 mL/100 gbw) via a gastric tube, resulting in salt-induced hypertension. Plant extracts that were antihypertensive function in one of three ways: cardio depression, increased diuresis, or vaso relaxation. The elimination of oxidative stress because of plant extract was an essential factor to note Excessive salt use which was linked to an increase in the formation of harmful Reactive oxygen species (ROS) in tissues. Bopda et al. observed that flavonoids and polyphenols presence was in favour of antihypertensive property (21).

ANTI-DIABETIC
Anti-diabetic properties of Bryophyllumpinnatum (aqueous extract) leaves were relevant. The diabetic treating activity of aqueous extract was compared with study comprising a blend of the extract and glibenclamide, a commercial diabetic medication. The experimental diabetic rats were given a specific dose of the leaves of Bryophyllumpinnatum (aqueous extract), and their BGL was measured and noted. When Bryophyllumpinnatum
leaves were used only, within 120 minutes the diabetic rats' BGL dropped significantly similar to natural level of blood glucose (22).

The active constituents in the anti-diabetic action were quercetin and kaempferol. The ethyl acetate fraction of *B. pinnatum'*s partitioned methanolic extract reduced BGL in alloxan-induced diabetic rats that inhibited amylase and glucosidase activity having IC50 values (137.89 and 110.15 µg/mL) respectively. Anti-diabetic property of leaves (*Bryophyllum pinnatum*) had been vastly documented, and the studies supported the assertion in both animal models i.e. in vitro and in vivo models. Ibitoye et al. had reported the active constituents in the anti-diabetic action were quercetin and kaempferol (23).

**ANTI-HEPATOTOXICITY**

The sleeping time after a given dosage had been developed as an indicator of hepatic metabolism since barbiturates were almost entirely metabolized in the liver. The ability of the *K. pinnata* concentrate and ethanolic extract to shorten the duration of thiopental-induced sleep in CCl4 rats was further evidence of the test substances' antihepatotoxic capacity.

The plant concentrates reduced serum bilirubin levels by 105.5 percent, and the concentrate and ethanolic extract reduced SGPT levels by 92.4 and 81.3 percent, respectively. Since GPT was more specific than GOT as a marker of hepatic injury, these findings clearly support the drug's major hepatoprotective role (24). Ngobidi et al. reported the presence of flavonoids and phenol compounds had hepatoprotective effects which were due to its antioxidant property (25).

**NEPHROPROTECTIVE**

In a rat model of ethylene glycol-induced urolithiasis, the nephron protective impact of *Bryophyllum pinnatum* synthesized silver nanoparticles (AgNPs) was estimated. The process of AgNPs was environmentally safe and non-toxic and their scale have an important role in increasing reactivity surface area thus reducing size and quantity by high bioavailability Dighade et al. had reported that active constituents were saponins, polyphenols and flavonoids which contributed to nephron protection (26).

II. PHARMACOLOGICAL ACTIVITY OF STEM

**ANTIMICROBIAL**

The antimicrobial activity findings revealed that the methanol stem extract was having strong antimicrobial activity against the S. aureus and *B. subtilis* (P <0.01). At the concentrations of 25.0 mg/ml and above, the aqueous extract of stem substantially (P< 0.01) demonstrated antibacterial activity against *Salmonella typhi* and *Bacillus subtilis* when the concentration was 12.50 mg/ml, fractions of methanol and aqueous extracts had the least antibacterial activity for *S. aureus* and *B. subtilis*, respectively. Nwadingwéhad reported alkaloids, resins, steroids, saponins, flavonoids, glycosides, and terpenoids for antibacterial activity (27).

**ANTIOXIDANT**

The maximum concentrations of polyphenols were found in methanol stem extracts (32 ± 1.2 mg GAE/g), respectively. Although alkaloids have the lowest concentration (0.03 ± 0.02 mg/gm) in methanolic extract of stem flavonoids prevent free radicals from oxidising low density lipoprotein (LDL). Flavonoids shows antioxidant property because they attack the free- radicals, prevents lipid peroxidation process and metal chelating activity (28). The identification of resulted stigmasterol bands or patterns in the sample chromatogram was verified using a picture of a thin layer chromatography plate (TLC) after performing stigmasterol chromatography pattern (29). Stigmasterol causes decrease in lipid peroxidation in the hepatic part and increase in the activities of catalase, superoxide dismutase (30). Daniel et al. had reported flavonoids as major constituent for antioxidant potential (28).

**ANTICONVULSANT**

The methanolic stem extract increased latency of tonic clonic seizures, myoclonus, and or clonus convulsions in a dose-dependent manner, functioning more likely as diazepam and also providing 100% defence from the fatal effects of pentylentetrazol. Mora-Perez et al. had reported terpenes and sterols for anticonvulsant activity (31).

III. PHARMACOLOGICAL ACTIVITY OF ROOTS

**ANTI-INFLAMMATORY**
When comparing with the reference drug diclofenac sodium, aqueous extract (87.46 percent) was found to be the most efficient when tested in vitro during anti-inflammatory research with an IC50 value of 570.24 μg/ml. The most effective inhibitor of xanthine oxidase production in vitro was found to be methanol extract (88.24 %). Polyphenols were a diverse and plentiful group of secondary metabolites that have been shown to have anti-inflammation action, which means providing arthritis defence. Latif et al. had reported polyphenols for anti-inflammation potential in stem (32).

**URIOLITHIATIC PROPERTY**

By virtue of their Ca²⁺ chelating capacity, *B. pinnatum* root extract was found to reduce the availability of calcium in renal tubules, preventing aggregation of already formed CaOx crystals and subsequent stone formation. The highest inhibition of CaOx crystal forming and aggregation in urolithiasis was found in the following order: leaf > stem > root. Nagarajan Y et al. had reported that the presence of steroid, terpenoids, and alkaloids were compounds behind anti-uroliithiasis activity (33).

**ANTICONVULSANT**

Methanolic Root Extract improved the abeyance of seizures (tonic-clonic) which was proportional to the dose in an inverse manner, and had relatively same effect on the lethal results of pentylenetetrazol. Mora-Pérez et al. observed that sterols and alkaloids present in methanol fraction to be responsible for the anti-convulsion action (31).

**ANTIBACTERIAL**

*Kalanchoe pinnata* roots were extracted using methanol, chloroform, aqueous and petroleum ether solvent and tested against *S.aureus, E.coli, P.aeruginosa*, and *C.albicans* for antimicrobial activity (in vitro). When comparison was done to other antibacterial extracts, it was found that methanolic extract of *Kalanchoe pinnata* roots was found to be the most effective. None among the extracts had any effect on Candida Albicans (34). Biswas at al. had reported that flavonoids were responsible for the antibacterial activity (35).

**ANTIHELMINTIC**

Antihelmintic activity against *Ascardiagalli* and *P. posthuma* (Annelida) was tested in vitro (nematode). The researchers reported that methanol, chloroform, and the aqueous extracts of *Bryophyllum pinnatum* had significant anthelmintic efficacy, whereas petroleum ether did not had any, when compared to the references, Piperazine. The roots extract of *Bryophyllum pinnatum* induced paralysis and death of the worms, particularly at higher concentrations like 100 mg/kg. Majaz AQ et al. had reported tannins for anti-helmentic potential (36).

**TOXICITY STUDIES**

Adult male or female mice weighing between 20 and 30 gm mice in each group was splitted into five groups of ten mice. Each mice given the same dosage of *Bryophyllum pinnatum* aqueous extract intraperitoneally at doses of 50 to 1000 mg/kg of body weight. 24 hours later, the count of deaths occur in each category the percentage mortality rate was determined after the data was collected and the LD50 was calculated by plotting the log dose against the percentage mortality. *B. Pinnatum* aqueous extract has no serious toxicity (LD50 = 660.90 ± 2.65 mg/kg of body weight) (37).

**IV. CONCLUSION**

The plant was used from years for the treatment and prevention of diseases for its antihypertensive property, burns, wound healing and many other medicinal purposes. The whole plant including leaf, roots, stem were enriched with bioactive compounds and needs to be evaluated in scientific manner using specific animal models and clinical studies were needed in order to get more valuable pharmaceutical formulations from this interesting plant. We hope we should try to screen this plant for treatment of various other diseases and develop suitable formulations to be used for mankind in future.

**REFERENCES**


www.turkphysiotherrehabil.org