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Pharmacological AMD toxicological studies of crude extract of impatiens *Balsamina linn*

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Abstract

Impatiens balsamina belongs to the family balsaminaceae, is an annual plant commonly known as 'garden balsam' or 'rose balsam'. This plant is native to southern Asia in India. *I. balsamina* is used in traditional methods such as Ayurveda, Unani and Siddha for various diseases and physiological conditions such as Jaundice, Corns, Snake bite etc. Phytochemical studies revealed the presence of many valuable compounds like naphthoquinones, coumarins, phenolic acids, flavonoids, anthocyanidins and steroids. The different parts of the plants like leaves, stem juice,flower are used in different places. *I. balsamina* have been reported to have various pharmacological activities such as antibacterial, antimicrobial, antifungal, analgesic, anti-inflammatory, antioxidant, antipruritic effects. The current review summarizes published information about the ethanopharmacology, chemical constituents, biological activities and toxicological study I.balsamina. The present review summarizes all the research work carried out on this plant in order to provide updated information for future works.

Keywords: Phytochemical studies; Pharmacological activities; Impatiens balsamina; Ethanopharmacology; Chemical constituents

1 Introduction

Traditional medicine (also known as indigenous or folk medicine) comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as:"the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Plant derived drugs have been a part of the evolution of human, healthcare for thousands of years. Plantbased drugs were commonly used in India. *Impatiens balsamina* is a species of Impatiens native to southern Asia in India.It belongs to the family of Balsaminaceae. The family consist of more than 1,000 species, but only two genera are recognized. It is an annual plant growing to 20–75 cm tall, with a thick, but soft stem.The leaves are spirally-arranged, 2.5–9 cm long and 1–2.5 cm broad, with a deeply toothed margin. The flowers are pink, red, mauve,lilac, or white, and 2.5–5 cm diameter; they are pollinated by bees and other insects, and also by nectar-feeding birds. The ripe seed capsules undergo explosive dehiscence Synonyms of *Impatiens balsamina* is *Impatiens coccinea, Impatiens corneta, Balsamina hortensis*. The balsams have a short life cycle, large flowers, and rather precise differentiation of color classes.

Impatiens balsamina (English name-Rose balsam, Hindi name – (Gul mehendi). locally known as balsam in Kerala belonging to the family Balsaminaceae The leaves are simple, alternate, ovate-lanceolate and serrate. Many compounds

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have been isolated from *I.balsamina*L.,including phenolics,flavonols ,anthocyanin pigments, and saponins . The genus Impatiens is richin organic acids, anthraquinones and flavonoids. the isolation of three monoglucosides of kaempferol, quercetin and pelargonidin from the stem of *I. balsamina.* Similarly salicylic acid, sinnapic acid, cafeic acid, scopletin, 2-hydroxy, 1,4-naphthoquinone and 2-methoxy 1,4-naphthoquinone had been extracted and purified from the stem of I. balsamin also isolated a new biscoumarin, 4, 40-biisofraxidin, from the roots of *I. balsamina*. [31] .Although the cell cultures were capable of producing coumarin derivatives, scopoletin and isofraxidin, the antifungal naphthoquinones were not be detected. This work was focused on using 2-methoxy-1,4-naphthoquinone high yielding plants as the initiated material for the establishment of the naphthoquinone producing cell cultures.*I.balsamina* could be used to remove naphthalene as the organic contaminant in the contaminated soil.Colchicine treatment increased plant height, stem circumference, leaf length and number of branches.

Different parts of the plant are used as traditional remedies for disease and skin afflictions. Juice from the leaves is used to treat warts and snakebite, and the flower is applied to burns. The extracts of *I. balsamina* also showed a long lasting skin moisturizing effect and prevent dryness, rough skin chap, dandruff and splitting hair ends, hence are used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents .Different parts of the plant are used to treat disease and skin afflictions; the leaves, seeds and stems are also edible if cooked. *Impatiens balsamina* L. has been used as indigenous medicine in Asia for the treatment of rheumatism, fractures and fingernail inflammation. Modern chemical and pharmacological studies have identified flavonol and naphthoquinone derivatives, some of which have strong antimicrobial, anti-anaphylaxis, anti-inflammatory as well as itch alleviating and anti-dermatitis activities, as the main chemical components of this plant. The seeds of this plant are edible. Alcoholic extract of the flowers has been found to have adequate antibiotic activity for against scleroting, fructicolaand other pathogenic fungi and bacteria. It is reported to be useful for pains in the joints. The seeds of *Impatiens balsamina linn* were extracted with respective solvents. The successive seed extracts of the plant were tested for their antimicrobial activity. The seed extract in various solvents have been found to possess promising antibacterial and antifungal activities .The plant has been reported to have various pharmacological activities such as antibacterial, antimicrobial, antifungal, analgesic. Antioxidant , anticancer ,antitumor, anti-inflammatory, antipruritic, antidermatitic acute toxicity. mosquito larvicidal activity. This review intent to summarize diverse studies on this plant and critically evaluates the issues associated to ethanomedicinal uses, phytochemistry, pharmacology and toxicology of *Impatiens balsamina.*

1.1 Phytoconstituents

Various flower colors exhibited in this species have been ascribed previously to the presence of glycosides of three anthocyanidins: pelargonidin, peonidin and malvidin, but no association of the pigment content with the genetic structure of the plant was made in these studies. The species is one of a family of herbaceous plants which is known to produce leucoanthocyanins and flavonols hence an intensive genetical and biochemical study may reveal the metabolic interrelations of several classes of similar compounds.

Induction of naphthoquinone formation in *Impatiens balsamina* cell cultures was achieved by using parent plants with high yielding of 2-methoxy-1,4-naphthoquinone as initiated explants. The cell culture with red-yellowish color was established in B5 medium supplemented with 0.1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 mg/l 6-benzylaminopurine (BA). The cell cultures were capable of producing two naphthoquinones, lawsone, and an unknown, which was more polar than lawsone. The time-course of growth and lawsone production in *I. balsamina* cell culture were also established *Impatiens balsamina* L., Balsaminaceae) has long been used in Thai traditional medicine . Several groups of secondary compounds including naphthoquinone, coumarin derivative, flavonoid and steroid have been reported from this species . Naphthoquinones, lawsone and 2-methoxy-1,4-naphthoquinone, were found to be a group of the main constituents exhibiting antifungal and antibacterial activities. The establishment of *I. balsamina* cell cultures in order to study secondary metabolite production has, been reported previously . Although the cell cultures were capable of producing coumarin derivatives, scopoletin and isofraxidin, the antifungal naphthoquinones were not be detected. This work was focused on using 2-methoxy-1,4-naphthoquinone high yielding plants as the initiated material for the establishment of the naphthoquinone producing cell cultures.

Ethylacetate extracts showed higher free radical scavenging capacity and phytochemical analysis revealed the presence of alkaloids, tannins, steroids, saponins and flavonoids. The groups of compounds commonly found in this plant are naphthoquinones, coumarins, phenolic acids, flavonoids, anthocyanidins and steroids.The naphthoquinones, lawsone, lawsone methyl ether and methylene-3,3'-bilawsone are a group of pharmacological active compounds.

The compound identified as (3,5,7 trihydroxy-2-(3,4 dihydroxyphenyl-4H chromen-4-one) Quercetin (C15 H10O7)(11). Compounds of 2-methoxy-1,4-naphthoquinone (MeONQ) and stigmasta-7,22-diene-3β-ol (spinasterol) were isolated from the pods and roots/stems/leaves of *I. balsamina* L., respectively. The active compounds isolated

from this plant include peptides (Ib-AMP1-4) from seeds, quinones[1, 4-naphthoquinone, lawsone, 2-methoxy-1,4-naphthoquinone (MeONQ), balsaquinone, impatienol,naphthalene-1,4-dione] from petals, pericarp and aerial parts, and flavonoids (kaempferol, quercetin, rutin, astragalin, nicotiflorin, naringenin and their derivatives) from petals and leaves.

The naphthoquinones lawsone, or hennotannic acid, and lawsone methyl ether and methylene-3,3'-bilawsone are some of the active compounds in *I. balsamina* leaves. It also contains kaempferol and several derivatives. Baccharane glycosides have been found in Chinese herbal remedies made from the seeds .A novel natural bisnaphthoquinone, methylene-3,3′-bilawsone, was isolated from root cultures of Impatiens balsamina, along with two naphthoquinones (lawsone and 2-methoxy-1,4-naphthoquinone), two coumarin derivatives (scopoletin and isofraxidin) and a sterol (spinasterol).

2 Materials and Methods

2.1 Materials

Test Drug- *Impatiens balsamina*

- Animals Used-
  - For acute Oral Toxicity Study (OECD 425)
    - No. of mice-06
    - Sex- Both Either (Male/Female)
    - Strain- Albino Wistar Rats
    - Weight- (150 – 200 g)
  - For In-Vivo Study
    - No. of Rats- 24
    - Sex- Male / Female (either)
    - Strain- Albino Wistar Rats
    - Weight- 150-200gm
- Diet- Food pellets consumed by Rats.
- Housing Conditions-
  - In separate cages 12-12hour light and dark cycle
  - Relative Humidity - 40-60%
  - Temperature - 25˚C (±2˚C)
- Other Requirements-
  - Glassware's- Beaker, Glass rod, Measuring Cylinder, Petri-dish, Test-tube, Pipette, Micropipette, Butter paper.
  - Chemicals- Ethanol, Chloroform, CMC, Sodium Hydroxide, Hydrochloric Acid, Sulphuric acid, Benzene, Iodine, Lead Acetate, Magnesium Turnings, Nitric Acid, Gelatin, Ferric Chloride, Sodium Chloride.
  - Other- Oral feeding Needle, Cotton, Markers, Eppendorf tube, Gloves, Marker pen, Syringe-5 ml etc.
  - Instruments–
    - High precision balance
    - Desiccator
    - Hot air oven
    - Incubator
    - Sonicator
    - Soxhlet apparatus
    - UV spectroscopy
    - Hot air oven

Others- Oral feeding needle, Eppendorf tube, Capillary tube, Dispovan syringe, Cotton, Marker pen.

2.2 Methods

2.2.1 Preliminary work (Selection of Plant):

The plant was selected on the basis of their antioxidant and antimicrobial activities and wide medicinal uses in the traditional literature. The ease of availability of plant is also taken in to consideration during selection.

Gathering sufficient information from vivid articles and journals it was concluded that there is scope to explore some more pharmacological activities in the plant ***Impatiens balsamina*****,** hence it was selected for further studies.

2.2.2 Collection of plant

Impatiens balsamina was collected from (M.P.) during the month of Sept. 2018.

2.2.3 Drying, size reduction and storage of plant material

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

2.2.4 Preparation of Impatiens balsamina Stem and leaves (L.)extract

- Extraction of ***Impatiens balsamina*** was done by Soxhlet extraction method.
- **Soxhlet Extraction:** Soxhlet apparatus was used for the extraction and hydro-alcoholic solvent (1:1) was selected as a solvent for extraction and calculated percentage yield of the extract.

Figure 1 Extraction Process (Soxhlet apparatus)

2.2.5 Screening of powder (Physiochemical Analysis)

Physiochemical screening of powdered leaves was done by the standard reported methods.

- Loss on Drying:
- Total Ash Value:
- Acid Insoluble Ash Value:
- Water Soluble Ash Value
- Foaming Index:

2.2.6 Phytochemical Analysis of Crude Extracts [Kokate CK, Khandelwal KR,1997]

The crude extracts of plants obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein etc.

Tests for Alkaloids

- Mayer's test: 1ml of each extract was mixed with a few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Formation of cream color precipitate indicates the presence of alkaloids.
- Wagner's test: To 1ml of each extract was mixed with equal volumes of Wagner's reagent (Iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

- Dragendorff's reagent test: To 1ml of each extract, 2 ml of Dragendorff's reagent was added and mixed. To this 2 ml of dilute HCl was added. Formation of an orange colored precipitate indicates the presence of alkaloids.
- Hager's test: To 2ml of each extract, a few drops of Hager's (Saturated picric acid solution) reagent were added. Formation of a bright yellow colored precipitate indicates the presence of alkaloids.
- Tannic acid test: A pale yellow- brown colored precipitate obtained when the extracts are treated with 10% tannic acid conforms the presence of alkaloids.
- FeCl3 test: To 1-2 ml of all the extracts, add few drops of neutral ferric chloride solution. Deposition of yellow precipitate indicates the presence of alkaloids.

Tests for glycosides

- Raymond's test: The extracts when treated with dinitrobenzene in hot alkali, pink to violet color will be observed indicating the presence of glycosides.
- Legal's Test: The extracts when treated with pyridine and alkaline sodium nitroprusside solution was added, appearance of cherry red color indicates the presence of glycosides.
- Bromine Water test: The extracts when treated with bromine water yielded a pale yellow color indicates the resence of glycosides.
- Keller Killiani test: 1ml of the extracts were dissolved in 1ml of glacial acetic acid and cooled, after cooling, 2-3 drops of ferric chloride was added. To this solution 2ml of conc. H2SO4 was added carefully along the walls of the test tube. Appearance of reddish brown colored ring at the junction of two layers indicates the presence of glycosides.
- Conc. H2SO4 test: To 1ml of the extracts, 1ml of conc. H2SO4 was added and allowed to stand for 2 min. a reddish color precipitate indicates the presence of glycosides.
- Molish's test: 2-3 drops of molisch reagent was added to the extracts and mixed well. To this, a few drops of conc. H2SO4 was added carefully. Formation of reddish-purple colored ring at the junction of two layers indicates the presence of glycosides.

Test for Quinones

- The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

Test for Saponins

- 5ml of each extract is taken in a test tube and shaken vigorously to obtain a stable froth. To this frothing solution, 5-6 drops of olive oil was added. Formation of an emulsion indicates the presence of saponins.

Tests for Phenols

- Ellagic acid test: The extracts were treated with few drops of 5% (w/v) glacial acetic acid followed by 5% (w/v) $NaNO_2$ solution. Formation of muddy brown color indicates the presence of phenols.
- Phenol test: 2ml of the extracts were separately treated with 1ml of FeCl3 solution. Development of an intense color conforms the presence of phenols.

Tests for Tannins

- Ferric chloride test: The extracts were treated separately with few drops of FeCl3 solution. Formation of blackish precipitate indicates the presence of tannins.
- Gelatin test: The extracts were treated with few drops of gelatin solution. Formation of white precipitate indicates the presence of tannins.
- Lead acetate test: To 1-2 ml of extracts, basic lead acetate was added separately. Formation of bulky red precipitate indicates the presence of tannins.
- Alkaline Reagent test: 1-2 ml of extracts were treated with a solution of sodium hydroxide. Appearance of yellow to red color indicates the presence of tannins.
- Mitchell's test: The extracts when treated with a solution of iron and sodium tartarate gives a water soluble, ammonium citrate insoluble complex indicating the presence of tannins.

Tests for Flavonoids

- Zinc-HCl reduction test: To all the extracts add a pinch of Zinc dust and a few drops of Conc. HCl. Formation of deep red color indicates the presence of Flavonoids.

- Lead-acetate test: To 1-2 ml of all the extracts, add few drops basic lead acetate solution. Formation of reddish brown precipitate indicates the presence of flavonoids.
- Shinoda's test: To 1-2 ml of all the extracts, add a small piece of magnesium paper and add a few drops of conc. HCl carefully along the walls of the tube. Appearance of red color indicates the presence of flavonoids.
- FeCl3 test: To 1-2 ml of all the extracts, add few drops of neutral ferric chloride solution. Deposition of blackish red precipitate indicates the presence of flavonoids. E.Alkaline Reagent test: to 1-2 ml of all the extracts, a solution of sodium hydroxide is added. Appearance of yellow to red color indicates the presence of flavonoids.

Test for Sterols

- Liebermann-Burchard test: To 1-2 ml of all the extracts, a few drops of acetic anhydride solution was added. To this mixture, a few drops of Conc. H2SO4 was added carefully along the walls of the test tube. Formation of reddish brown ring at the junction of two layers indicates the presence of steroids.
- Salkowski test: to 1-2 ml of all the extracts, 5ml of chloroform was added. To the above mixture, 1ml of conc. H2SO4 was added carefully along the walls of the tube and mixed. The formation of reddish color in the lower layer indicates the presence of steroids.

Test for Terpenoids

- To 1-2 ml of all the extracts 1% HCl was added and allowed to stand for 5-6 hours. Later, these extracts were treated with 1ml of Trim-Hill reagent (a solution of 10 ml of acetic acid, 1 ml of 0.2% copper sulphate in water and 0.5 ml of concentrated hydrochloric acid) and heated in a boiling water bath for 5-10 minutes. Formation of bluish green color indicates the presence of terpenoids.

Tests for Carbohydrates, gums and mucilage's

- Benedict's test: To 5ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of red precipitate indicated the presence of sugars.
- Molisch's test: A small fraction of extract was taken in ethanol separately and a few drops of 20% w/v solution of α-napthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates.
- Fehling's test: Extract heated with dil. HCL than neutralized with NaOH than added fehling's solution A & B. Brick red precipitate was formed. It's indicated the presence of carbohydrates.
- Test with 95% alcohol: When 95% alcohol added to the extract, gums get precipitated out. The precipitate is insoluble in alcohol.
- Ruthenium red test: In this test 0.08 gm of ruthenium red dissolved in 10 ml of 10% solution of lead acetate, it stains the mucilage to red color.

Test for Proteins

- Biuret test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.
- Acute oral toxicity studies (OECD 423) (54)
- Acute oral toxicity study was evaluated as per OECD guidelines (423) on Wistar albino rats. Before experimentation rats were fasted overnight with water ad libitum. The crude extracts were suspended in a vehicle (hydro-alcoholic extract of plants extract+ 1% aqueous CMC by gavage using oral cannula). The first four groups received 10, 100 ,1000 and 2000 mg/kg body weight of the HAIB to rats. Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found).
- Procedure and Observation
- Male and Female Albino rats of weighing 150-200g were used for the study. These were acclimatized to laboratory condition for one week prior to start of dosing. Hydro-alcoholic extract of Impatiens *balsamina linn* was dissolved in suitable solvent (1% aqueous CMC), to prepare dose of 10, 100, 1000 and 2000 mg/kg. The doses were selected according to the OECD guideline no. 425. The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy rats (each set of 3 rats) were used for this experiment. First set of animals were divided into four groups, each of three in a group.

- Animals were fasted overnight with water ad libitum. Animals received a single dose of 10, 100, 1000 and 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of HAIB, food was withheld for 3-4 hrs. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD50 is greater than 2000mg/kg, if three or more animals survive. If an animal unexpectedly dies/late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors [OECD 200.1-guideline].

Table 1 Animals were grouped as follows for Sub acute oral toxicity studies

Group	Name of Group	Treatment
1	Control	Normal saline (5 ml/kg p.o. body weight)
2	Test-1	*HA**IB*** 10 mg/kg p.o. body weight)
3	Test-2	*HA**IB*** (100mg/kg p.o. body weight)
4	Test-3	*HA**IB*** (1000 mg/kg p.o. body weight)
5	Test-3	*HA**IB*** (2000 mg/kg p.o. body weight)

Where:- *HAIB* = Hydro-alcoholic extract of *Impatiens balsamina*

2.2.7 Sub acute oral toxicity studies

About 24 albino rats were randomly grouped into 4 (control, Test-1, Test-2 and Test-3) of four rats each. Group A served as control and was administered with 0.5 mL of normal saline once daily for 28 days. Rats in groups Test-1, Test-2 and Test-3 were orally gavaged with 100, 300 and 600 mg/kg body weight of the aqueous and methanol extracts, respectively once daily for 28 days. The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period.

Table 2 Animals were grouped as follows for Sub acute oral toxicity studies

Group	Name of Group	Treatment
1	Control	Normal saline (5 ml/kg p.o. body weight)
2	Test-1	*HA**IB*** 100 mg/kg p.o. body weight)
3	Test-2	*HA**IB*** (300 mg/kg p.o. body weight)
4	Test-3	*HA**IB*** (600 mg/kg p.o. body weight)

Where:- *HAIB* = Hydro-alcoholic extract of *Impatiens balsamina*

2.2.8 Termination of the experiment

On the 29th day of the protocol, following an overnight fast of 8 h, all animals in various groups were anesthetized under chloroform and blood samples were collected into non-heparinised and heparinised bottles for haematological and biochemical investigations respectively. Blood samples collected into clean non-heparinised bottles were allowed to clot and centrifuged according to groups; and serum was separated from the clot into clean bottles for the biochemical analyses. The liver, kidneys and heart were excised from dissected rats, immediately cleaned of blood by using physiological saline and weighed. The liver and kidneys were then fixed in 10% formalin for and use for the histopathological examination.

2.2.9 Calculation ratio of organ-to-body weight

Organ-to-body weight ratio was calculated by dividing the weight (gm) of each organ by the weight (gm) of rats before sacrifice.

2.2.10 Biochemical analyses

Commercial kits from Randox Laboratories Lmt, U K and Agappe Diagnostics were respectively used for the assay of liver and kidney indices.

2.2.11 Histopathological examination

The liver and kidneys excised from all the experimental rats were fixed in 10% buffered formalin in labeled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 mm thick, stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope.

3 Result and Discussion

Results shows that compound 2 has more fungicidal effect, followed with compound 1 and 3. Compound 2 is active against gram positive and gram-negative aerobic bacteria. Compound 3 showed antibacterial effect against S. epidermidis, B. subtilis and not active against S. aureus, and E. coli. Compound 1 also active against all the tested microbes.

3.1 Antipruritic and antidermatitic activity

- **Methodology:** Four-week-old NC mice with no symptoms were administered with 100 mg/kg/day of Impatiens balsamina until 13 weeks old. Dermatitis was evaluated by placing mice in polysulphonic cage under standard laboratory conditions for 2 days and observing their scratching behavior for 20 min. As a negative control C3H mice was used.
- **Results**: Results shows that 35% ethanolic extract of Impatiens balsamina reduced the pruritic effect in NC mice with dermatitis.

3.2 Biological activity of oleanane -type triterpenoidal glycosides

- **Methodology**: Assessment of NO generation and cell viability BV-2 cells were seeded in 96 -well plate and incubated in the presence of the test compound and lipopolysaccharides for 1 day. Produced NO2 was evaluated with Griess reagent. Absorbance was measured against 570nm.MTT assay was used for cell viability study.
- **Results**: Results shows that compound 1 has showed higher cell viability followed with compound 2 .

3.3 Antinociceptive activity of methanol extract of flowers

- **Methodology**: Acetic acid induced writhing test [15] World Journal of Biology Pharmacy and Health Sciences, 2022, 12(02), 054–060 56 The mice were treated with test drug or extract and then with 0.7% acetic acid after 15 and 30 min respectively at the dose of 10ml/kg body weight. Number of writhing were counted for 10 min after acetic acid treatment.
- **Result**: Oral administration of the extract causes reduction in writhing as compared to control group.200 and 400 mg/kg doses shows better antinociceptive activity.
- **Methodology**: Hot plate test Paw licking behavior of mice were observed for 15 s before and after drug treatment and compared with that of control groups.
- **Results**: 200 and 400mg/kg doses shows significantly increased reaction time.
- **Methodology**: Tail immersion test The latency between tail immersion and withdrawal was observed at 30,60,90 and 120 min of extract treatment.
- **Result**: The extract shows significant increased latency period at 100,200 and 400 mg/kg doses.
- **Methodology**: Formalin test Mice were injected with 20µl of 1.35% formalin into sub planar region of right paw 30 min after extract treatment and 15 min after injection of morphine. Licking of injected paw was observed at 5,15 25 min after formalin injection.
- Result: Extract treatment reduces formalin induced paw licking behavior at 100,200 and 400 mg/kg doses.
- **Methodology**: Hole cross test - A cage with fixed partition having a hole of 3cm diameter was used. The number of passages of the mice through the hole from one chamber to other was counted for a period of 3 min, at 0, 30, 60, 90 and 120 min after the treatments.
- **Result**: The extract did not produce significant decrease of movement in comparison to control group in the doses at 100,200 and 400 mg/kg at 60 min. However significant decrease in movement was produced at 90 and 120 min.
- **Methodology**: Open field test - The number of squares visited by the mice was counted for 3 min at 0,30,60,90 and 120 min after treatment.

- **Result**: Significant inhibition of locomotion was produced at 100,200 and 400 mg/kg doses. World Journal of Biology Pharmacy and Health Sciences, 2022, 12(02), 054–060 57
- In-vitro antidiabetic and anthelmintic activity of hydro alcoholic extract of Impatiens balsa mina roots:
- **Methodology**: In-vitro antidiabetic activity • Alpha-amylase inhibitory activity Reaction mixture containing 50µl phosphate buffer (100mM, pH = 6.8), 10µl α–amylase (2U/ml), and 20µl of varying concentrations of extract (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was pre incubated at 37°C for 20 min. Then, the 20µl of 1% soluble starch (100mM phosphate buffer pH 6.8) was added as a substrate. Incubated further at 37°C for 30 min; 100µl of the DNS color reagent was then added and boiled for 10 min. The absorbance was measured using photo colorimeter at 540 nm. Acarbose was used as a standard. Without test (extract) substance was set up in parallel as control and each experiment was performed in triplicates . The results were expressed as percentage inhibition.
- **Result**: The extract shows antidiabetic activity

3.4 In-vitro anthelmintic activity

Adult Motility Assay (AMA) AMA was conducted on 75 mature Pheretima posthuma worms. Test was performed in 5cm diameter glass Petridish . Three concentrations of plant extract were used. There were 5 groups as follows:

- Group I: Hydro alcoholic extract at10mg/mL
- Group II: Hydro alcoholic extract 25mg/ml
- Group III: Hydro alcoholic extract at 50mg/mL
- Group IV: Albendazole at 100mg (positive control)
- Group V: Water (negative control).

The inhibitions of motility of worms were used as indication of worm mortality or paralysis, and were observed till 7hr. post treatment. Worms not showing any motility were picked out and kept in lukewarm water at 40 ˚C for 10 minutes and, in case of revival in motility, the observed worms were counted as alive; otherwise, they were counted as dead.

- Result: The extract shows anthelmintic activity.

3.5 Antianaphylactic activity

- **Method**: Male ddY mice of 6 weeks were used for the study. They were sensitized subcutaneously on day 0 with 50µg of HEL emulsified in Freund's incomplete adjuvant. On day 9 mice were given 50µg of HEL i.v. HEL-sensitized mice in control group were challenged with bovine serum albumin.
- **Result**: The treated groups show decrease in rate of anaphylaxis and mortality when compared to control group at 256 mg/kg dose.

3.6 Antioxidant property

Method: DPPH scavenging assay - Extract at various concentrations was mixed with methanol and were added to freshly prepared methanolic solution of DPPH. Solution was allowed to stand for 30 min at room temperature and absorbance was measured at 517 nm. World Journal of Biology Pharmacy and Health Sciences, 2022, 12(02), 054–060 58 Reducing power assay

Extract in various concentrations were mixed with methanol. Later mixed with phosphate buffer (0.5 mL, 0.2M, pH 6.6) and potassium ferricyanide (0.5 mL, 1%). Incubate the above mixture at 50 0C for 20 min. Later add 0.5 mL of 10% (w/v) of trichloroacetic acid and centrifuge at 3000 rpm for 10 min. 1.5 ml of above solution was mixed with equal volume of distilled water and 0.1 mL of 0.1%(w/v) of ferric chloride. After 10 min measure absorbance at 700 nm. **Results:** DPPH scavenging assay Scavenging activity of diethyl extract was strongest, followed with methanol, chloroform and water extracts. Reducing power assay Diethyl extract showed significant reducing power followed by methanol, chloroform, water and petroleum ether extracts.

4 Conclusion

The present study was to detail about pharmacological amd toxicological studies of crude extract of impatiens *balsamina linn*. . The plant contains many useful phytoconstituents such as flavonoids, saponins, alkaloids, tannins, coumarins, and glycosides. The plant has many pharmacological actions due to the presence of these phytoconstituents. Traditionally, the leaves of garden balsam are suspected to contain poison that can affect the digestive system. Stem and leaf Methanol extract of garden balsam was fractioned into n- hexane fraction with liquid extraction method.Some of these

pharmacological actions are antimicrobial, anti-inflammatory, antipruritic, antidermatitic, antinociceptive, antineurodegenerative, antitumor, and antioxidant effect. Thus, this review can be used for future studies.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

References

[1] N Baskar;DB Parimala;B Jayakar, IJRAP.,2012,3(4),631-33.

[2] N Debashree; A Subhalakshmi ;S Rita ;A Pfuzia,Int J Pharm.Bio Sci., 2013,4(2),581-87.

[3] D Zhi-Shah;J Fu-Sheng ;C Ni-Pi;L Gui-Yuan Lv;Z Cheng-Gang,Molecules.,2008,13,220-229.

[4] EA Raliph;WH Charles; JR.Plant Research Institute, University of Texas, Austin, Texas and Department of Botany, Indiana University, Bloomington, Indiana. 1957,35-47.

[5] Y Chen; A Shinobu;O Hideaki, Balsaminaceae: 43-114.

[6] J Basanti, World Environ.,2011,6(2),299-300.

[7] ZN Mohd et al, Am-Euras.J.Agric.& Environ.Sci.,2014,14(7),610-14.

[8] G Marimuthu; R Mohan,JCLM., 2014,2(3),222-224.

[9] K Suk-Nam et al, Molecules.,2013 ,18,6856-65.

[10] P Pharkphoom. Qunatitative HPLC determination of napthoquinones in Impatiens balsamina leaves, Phytomedicine and Pharmaceutical Biotechnology Research Center, Faculty of Pharmaceutical Sciences.

[11] N Baskar; DB Parimala; B Jayakar, Int. J. Pharm.&H.CareRes.,2014,2(2),115-17.

[12] T Takeshi et al,Jpn. J. Food Chem.Safety., 2013,20(1),52.

[13] WS Benny;K Indri;NN Siti, Trad.Med.J.,2014,19(3),118-27.

[14] B Jitu,Rec Res Sci Tech.,2011,3,31-42.

[15] W Yuan-Chuen et al, Evidence-Based Complementary and Alternative Medicine., 2011,1-8.

[16] T Karin et al, Peptides, 2005, 26(7), 1113-1119.

[17] K Mari et al, Allergology International., 2007, 56, 113-123.

[18] B Archana;De Bratati,IJPPR., 2014,6(3),622-35.

[19] G Manish et al,International Journal of Phytotherapy Research.,2012,2(3),26-41.

[20] P Panichayupakaranant, Pharmaceutical Biology. 2001, 39, 1-4.

[21] S Athip;P Parkphoom, Natural Product Research: Formerly Natural Product Letters. , 2012, 26, 1119-1124.

[22] K Ishiguro, Phytother Res.,2011,15(6),506-510.

[23] SJ Ahmed;M Koperuncholan,IJPBS.,2012,3,401-406.

[24] F Xiaobo; S Holger; R Jürgen; Prof. Dr. W Michael,Biotechnology Journal.,2013,8(10),1213-1220.

[25] Herrera et al,Med. Plants Res.,2013,7(38), 2846-2858.

[26] I Kyoko;O Yukiko;O Hisae, J.Nat. Prod., 1998, 61 (9), 1126–1129.

[27] J Steven; et al,Systematic Botany.,2006,31(1),171-180.

[28] K Ishigurao;H Fukumoto; T Murashima; M Kuriyama; M. Semma; K. Isoi, Phytotherapy research., 2006, 6(2),112-113.

[29] D Wanchai et al, Phytochemistry.,1995, 40(4),1141-1143.

[30] SW Ni Made et al,Journal biology., 2011,(1),9-14.

[31] N Baskar et al,Int. jour.of Res.in Pharmacology and Pharmacotherapeutics.,2012,1(1),30-33.

[32] S Durre et al,J.Med. Plant. Res.,2010,(3),260-266.

[33] R Rajendran et al,A Journal of Science and Technology.,(2),60-64.

[34] Y Xiaolong et al,Phytother. Res. ,2001,676-80.

[35] B Shivaji et al,World Journal of Pharmacy and Pharmaceutical Sciences.,(2),5363-5376. [36] N Anwer et l,IFRJ.,2013, 20(1),99-103.