Phytochemical Evaluation, Pharmacological Activity and Toxicological Profile of Impatiens balsamina

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Review Article

ABSTRACT

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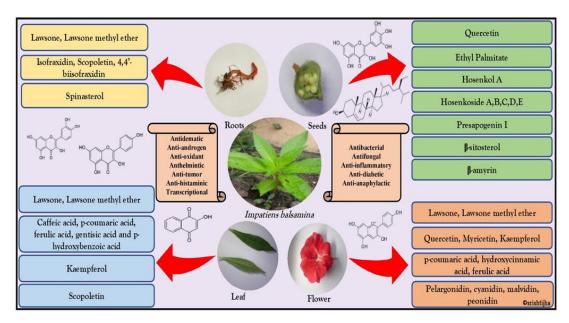
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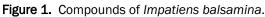
Impatiens balsamina (rose balsam) belonging to the family Balsaminaceae is an annual herb which is native to south east Asia. It has been used as cathartic, emetic, diuretic and has been used in treatment of wounds, inflammation of skin and jaundice. Various studies on this plant reveals the presence of flavonoids, naphthoquinone, quinones, triterpenoid, saponins, alkaloids and leucoanthocyanins. The plant and its different parts like seed, stem, leaf and flowers possesses various pharmacological activities like antifungal, antibacterial, antioxidant and antipruritic properties. This comprehensive review summarizes the phytochemistry, pharmacological activity and toxicological profile on Impatiens balsamina from various sources for future investigational works.

Keywords: Impatiens balsamina; Phytochemistry; Quercetin; Pharmacological activity; Toxicological profile

INTRODUCTION

Impatiens balsamina is an annual herb which has its distribution in south east Asian countries such as India, Myanmar, China, Indonesia ^[1]. It belongs to the genus Impatiens and family Balsaminaceae. It has been used in traditional system of medicines such as Ayurveda and Unani since time immemorial in treatment of scurvy, inflammation of nails and rheumatism ^[2]. The plant contains a variety of natural phytoconstituents such as flavonoids, coumarins, anthocyanidins, steroids and naphthoquinones which are being extracted from various parts like root, stem, seeds and leaves showing diverse pharmacological activities such as antimicrobial, antidiabetic, anti-inflammatory and anthelmintic (Figure 1).



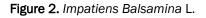


Botanical name: Impatiens balsamina L.

Family: Balsaminaceae

Synonyms: Balsamina angustifolia, Blum, Balsamina coccinea (Sims) DC, Balsamina cornuta (L.) DC, Balsamina foeminea gaertn, Balsamina hortensis Desp, Balsamina lacca Medik, Balsamina minutiflora Span, Balsamina mollis G. Don, Impatiens balsamina var. corymbosa Santapau, Impatiens coccinea Sims, Impatiens cornuta L, Impatiens eriocarpa Launert, Impatiens stapfiana Gilg.

Vernacular names: English: Balsam, Impatiens rose, spotted snap-weed and touch me not, Sanskrit: Dushparijati, Tairini, Hindi: Gul-mehndi, Manjrya, Phyaktuli, Timadia, Marathi: Gulmendi, Terada (Figure 2 and Table 1)^[3].





Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embrophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Asteranae
Order	Ericales
Family	Balsaminaceae
Genus	Impatiens L.
Species	Impatiens balsamina L.

Table 1. Taxonomical classification.

Geographical distribution:

- It is native to Western Asia, China, India, Sri Lanka, Myanmar and Malaysia.
- In Andhra Pradesh it is found in Kurnool, Vellore and Vishakhapatnam district.
- In Kerala it is found in Kannur, Wayanad, Kozhikode, Malappuram, Palakkad district, Thrissur, Idukki, Kottayam, Kottayam, Alappuzha, Pathanamthitta and Kollam^[4].

LITERATURE REVIEW

Cultivation and propagation

Soil: It grows in reasonably good heavy clay soil and loamy soil with high organic content ^[5]. It prefers moist and well drained humus rich soil ^[6].

Light: It can grow well in shade as well as in full sun. Usually, 6hrs of sunlight a day is well suited for their growth [7].

Temperature: It requires warm and moist conditions but cannot tolerate temperature below 300 Fahrenheit. It cannot tolerate frost.

Germination: The best suited germination temperature is 25-28°C.

Soil pH: It can grow well in acidic (<6.0), basic (>8.0) as well as neutral pH (6.0-8.0).

Altitude: It grows in altitude up to 3500 meters close to sea level.

Morphology

Impatiens balsamina is an annual herb with height ranging from 60-100 cm. The stem is robust, erect, succulent, simple or branched with 8 mm in diameter. The leaves are alternate and sometimes lowest one opposite. The petiole is 13 cm in length, adaxially shallowly sulcate with stipitate glands. The Leaf blade is lanceolate, elliptic or oblanceolate 4-13 x 1.5-3 cm, the lateral veins are present in pairs of 4-7. The inflorescence consists of 1 flower or 2-3 flowers fascicled in leaf axils without peduncles. The pedicels are 2-2.5 cm in dimensions, deeply public, bracteate at base with linear bracts. The flowers are white, pink, purple in color. The lateral sepals-2 ovate or ovate- lanceolate 2-3 mm. The Lower sepals 13-19 x 48 mm, deeply navicular, public, sheerly narrowed into incurved spur. The spur is slender 1-2.5 cm. The upper petal is

orbicular, retuse, mucronulate, and abaxial midvein carinate. The lateral petals are united shortly clawed 2.3-2.5 cm, bilobed. The basal lobes are obovate oblong and small. The distal lobes are suborbicular, apically retuse, auricule and narrow. 5 stamens are present. The filaments are linear. Anthers is ovoid, apex obtuse. The ovary is fusiform, densely pubescent. The capsule is broadly fusiform 1-2 cm, densely, tomentose, narrowed at both ends. Seeds are 1.5-3 mm, black-brown in color, globose and tuberculate ^[8].

Traditional uses

The plant is being used as an emetic, diuretic and cathartics ^[9]. It is useful in treating joint pain and used in treatment against warts ^[10].

- Leaves: Crushed leaves are used against skin inflammation and a combination of salt and castor oil is used to treat whitlow around fingers. It is also used to treat torn nails of fingers and legs.
- Flowers: The flowers are mucilaginous in nature and used as tonic. They have cooling effect when applied to burns. Snakebites can be treated from juice of the flowers ^[11]. The extracts from the flower shows antibiotic activity against fungi and viruses.
- Seeds: The seed is used as an expectorant and shows anticancer activity. Seeds in powdered form is given to women during labour pain in order to provide strength.
- **Roots:** The decoction obtained from the roots is used to treat irregular menses. The roots are used in treating of inflammation of the skin and nails that are torn (Figure 3).

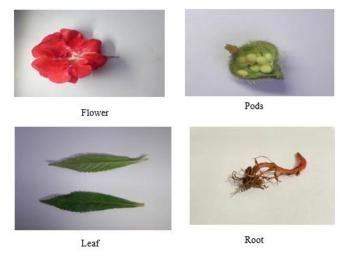


Figure 3. Parts of Impatiens balsamina L.

Phytochemistry: Many compounds such as flavanols, anthocyanins such as Cyanidin, Delphinidin, Cyanidin 3-O-glucoside, Pelargonidin and phenols are also derived from the plant ^[12]. The genus is rich in anthraquinones, flavonoids and some amount of organic acid. Three monoglucosides have been isolated from the stem that are quercetin, kaempferol and pelargonidin. Sinnapic acid, salicylic acid, scopletin, 2-hydroxy, 1,4-napthaquinone, biscoumarin-4 and 40-biisofraxidin are isolated from the roots. Cell cultures of the plant produces coumarins mainly isofraxidin and scopoletin (Figure 4 and Table 2).

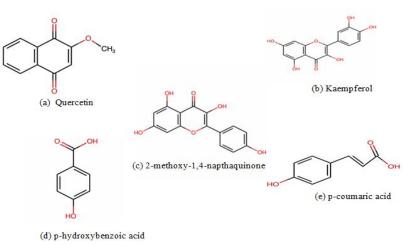


Figure 4. Some major chemical constituents present in Impatiens balsamina L.

Table 2. Some major chemical constituents present in various parts of Impatiens balsamina L.

Part	Category	Phytoconstituent	
		Kaempferol	
		Quercetin	
		Qcercetin-3-O- β -D-glucoside	
Stem	Flavonoid	Kaempferol-3-0- β -D-glucoside	
		Ethyl oleate	
		Ethyl stearate	
Seed	Fatty ester	Ethyl palmitate	
		β -sitosterol	
		β -amyrin	
Seed	Steroid	α-spinasterol	
Seeds	Flavonoid	Quercetin	
Seeds	Peptide	Ib-AMP 1,2,3,4	
		Lawsone	
Leaf	Naphthoquinone	Lawsone methyl ether	
		Kaempferol	
		Quercetin	
Flower	Flavonoid	Myricetin	

Pharmacological activity

Antibacterial and antifungal activity: The extract from the Impatiens balsamina were tested for significant activity against bacteria such as Staphylococcus aureus, Proteus vulgaris, Salmonella paratyphii, Shigella boydii and Cryptococcus neoformans. Its activity was also tested for fungal pathogens like Candida albicans. The extract showed high zone of inhibition in disc diffusion assay in all the above species except salmonella paratyphii and proteus vulgarius. The ethanolic extract of leaves show higher antimicrobial activity against clostridium perfringens and candida albicans. Moderate activity was observed in the case of *E. coli* and Salmonella typhimurium. Very slight activities were observed in the case of listera monocytogens. The three-naphthoquinone isolated from leaf extract were lawsone methyl ether, lawsone and methylene-3,3'-bilawsone. They show significant activity against facultative anerobic bacteria, yeast, dermatophyte fungi and aerobic

bacteria. Laws one methyl ether was proved to be most potent and showing broad spectrum of antimicrobial activity ^[13,14]. The extract from the leaves in concentrations such as 25%, 50% and 100% were showing similar activity to 2% ketoconazole in inhibiting the activity of Candida species. *In vitro* studies were performed against 7 bacterial and 5 fungal species to check the antimicrobial activity. The extracts were prepared from the roots and leaves using solvents such as hexane, alcohol, benzene, methane, chloroform, Pet. ether, methane, hot and cold water. The chloroform and ethanolic extracts possessed excellent antimicrobial activity ^[15]. 95% ethanolic extract yielded 2-methoxy-1,4-napthaquinone which shows activity against gram positive and gram-negative strains in all the fungi ^[16]. Kaempferol and quercetin, isolated from the flowers show activity against *propioni bacterium acnes* in concentration of 64 micrograms/ml ^[17]. The disc diffusion studies carried out by using seed extract shows antibacterial activity against *Bacillus anthracis* and *E. coli*. and antifungal activity against species such as *Fusarium* and *Aspergillus niger* ^[18].

Anti-inflammatory activity

The two new 1,4-napthaquinone sodium salts isolated from corolla show significant anti-inflammatory action by selective cyclooxygenase inhibition ^[19]. The roots and stem of *Impatiens balsamina* were washed, dried and coarsely grounded. They were divided into four equal proportions and extracted using ethanol and water for 6 days by cold maceration technique. The extract was collected and evaporated using rotary vacuum evaporator. The anti-inflammatory activity was screened using wistar albino rats (150-200 g) using carrageenan-induced paw edema method along with plethysmometer. 50mg/kg extract were administered to rats orally before carrageenan administration. The ethanolic extract of roots had the highest percentage yield (9.75%). The percentage yield of water extract of roots was 0.65%, ethanolic extract of stem was 5.86% and that of water extract of stem was 7.28%. The pathways involved in inflammation include cyclooxygenase pathway with prostaglandins E2 mediators and lipoxygenase pathway with Leukotriene B4 mediators. Diclofenac sodium inhibits the cyclooxygenase pathway which was given to animals in group 2. The anti-inflammatory activity of *Impatiens balsamina* can be attributed to the inhibition of lipoxygenase pathway ^[20].

Antidiabetic activity

The metabolites such as triterpenoids, tannins, flavonoids and anthraquinone glycosides are responsible for showing the antidiabetic activity. The antidiabetic activity of *Impatiens balsamina* was evaluated using hydroethanolic root extract in the concentration of 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml which showed 27.07%, 29.33%, 50.66%, 58.56% and 62.26% inhibition rates. Alpha amylase inhibition method was used to evaluate this activity. The IC₅₀ was observed at 0.316 mg/ml. Acarbose (IC₅₀ 0.206 mg/ml), an alpha glucosidase inhibitor was used as a standard that lowered the postprandial Type-II diabetes mellitus. It promotes delay in the absorption of glucose. Another study revealed that, extract prepared from the floral petals of wild balsam species showed antidiabetic activity due to the presence of anthocyanin. A dose dependent inhibitory effects were seen on the activity of enzymes like alpha-glucosidase in concentration of 200 µg/ml and alpha amylase in the concentration of 500 µg/ml. This inhibition was compared to standard acarbose in the concentration 189 µg/ml and 50 µg/ml.

Anti-anaphylactic activity

White petals from *Impatiens balsamina* were taken and 35% ethanolic extract was prepared from it. The extract shows antianaphylactic activity. Another study revealed that the ethanolic extract shows the presence of kaempferol 3-rutinoside and laws one which inhibited the decreased blood flow. The flower extracts from the plant possesses compounds with weak

antihistaminic action and work as platelet activating factor antagonist. This activity was determined in person with suffering from hypotension. The study was done using simulated anaphylaxis in mice.

Antidermatic/antipruritic activity

The pericarp of *Impatiens balsamina* yields balsaminones A and B which shows antipruritic activity. 35% ethanolic extract (100 mg/kg) obtained from the petals was used to study the activity using atopic dermatitis NC mice model. At a dose of 10 μ g/ml, the 2hydroxy-1,4-napthaquinone (Lawsone) and Kaempferol 3- rutinoside inhibited the scratching and dermatitis. The activity of mushroom tyrosinase was inhibited by the kaempferol obtained from the flowers. Melanin production is also inhibited from the streptomyces bikiniensis in dose dependent manner.

Anti H-pylori activity

The extract obtained from root, stem, leaf, seed and pod from *Impatiens balsamina* has significant bactericidal activity against H. Pylori. The extract from the pods was found effective which shows an MIC 1.25-2.5 and MBC 1.25-5.0 μ g/ml in agar dilution method. The ethyl acetate and acetone extract show significant activity. This activity is equivalent to amoxycillin. 2-methoxy-1,4-napthaquinone and stigmasta-7,22-diene- 3β-ol were examined for anti-H pylori activity. The MIC was 0.156-0.625 and MBC was 0.313-0.625 μ g/ml.

Anti-androgen activity

The impatienol and 3-hydroxy-2-(3-hydroxy-1,4-dioxo (2-napthyl))ethyl naphthalene-1,4-dione are bisnapthaquinone derivatives obtained from 35% ethanolic extract of ariel parts of Impatiens balsamina. Activity guided fractionation led to the identification of bisnapthoquinone derivative which shows testosterone α -reductase activity. Forty-eight male C57BL/6 mice were arranged into groups of blank, androgenetic alopecia model group, *Impatiens balsamina* group, *Lawsona inermis* L. group, Henna group and minoxidil group. The mouse was subjected to testosterone propionate solution 5 mg/kg to establish androgenetic alopecia. In *Impatiens balsamina* group and henna group the contents of testosterone and dihydrotestosterone were lower than those in model group (P>0.05).

Antioxidant activity

The antioxidant activity was evaluated by using ethanolic seed extract by three assay methods such as reducing power assay, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging method and phosphorous molybdenum assay. At 200 mg/kg concentration the ethanolic extract obtained from the entire plant showed *in vivo* antioxidant activity. Chromium induced oxidative stress in male albino rats was used as a method to determine the antioxidant activity. The aqueous extract of flower was evaluated to determine the anti-oxidant activity by 1,1-diphenyl-2-picrylhydrazl *i.e.*, DPPH radical scavenging (1140.36 μ g/ml) and Total antioxidant activity (13.04 μ g/ml).

Anthelmintic activity

Adult motility assay using 75 adults *Pheretima* posthuma worms was performed. Three different concentration of plant extract was prepared and poured into glass petridish. Group I contained hydroalcoholic extract at 10 mg/ml, group II-hydroalcoholic extract 50 mg/ml, Group-IV Albendazole at 100 mg a group V-water as negative control. The inhibition of motility of worms was an indicator for worm mortality. After 7 hours of treatment mortality was recorded. Worms not showing any activity were placed in lukewarm water and were checked for any chances

of revival and if no movement was observed they were counted as dead. Time of paralysis and time of death of *Pheritima* posthuma were recorded.

Anti-tumour activity

The chloroform and ethanolic extracts obtained from the leaves show significant *in vitro* antitumor activity against HepG2 carcinoma cell line. The separation and identification of the active constituents lead to the discovery of 2-methoxy-1,4-napthaquinone. This compound shows histoprotective effects on the pancreas, duodenum, stomach and spleen of tumor-induced mice. Using MTT assay (200 mg/kg dose) and Dalton's ascites lymphoma tumour bearing mice (400 mg/kg dose) the ethanolic extract was used to study the *in vitro* cytotoxicity and *in-vivo* anti-tumour activity by using NIH3T3 and Hela cells. It increases the life span and has protective hemopoietic activity. Thus, it shows cytotoxic effects and is used in cancer therapy. Balsaminone C, a dinapthonfuran-7,12-dione derivative shows cytotoxic activity against A549, Hela and Bel-7402 cancer cell lines. The cytotoxicity of 2-methoxy-1,4-napthaquinone against MKN45 gastric adenocarcinoma was identified. At dose higher than 50 µM serious necrosis occurred by using superoxide anion catastrophe method.

O-Methyltransferase activity

The o-methyltransferase enzyme in plant produces hydroxycinnamic acids and methylated anthocyanins. The red genotype shows low level of ferulic acid and methylated anthocyanins are absent from them, while the purple genotype shows high level of ferulic acid and methylated anthocyanins which relates to the o-methyl transferase activity.

Anti-platelet activating activity

Blood pressure monitoring system was used to study the platelet activating factor which is a chemical mediator of anaphylactic hypotension. Compounds isolated from 35% ethanolic extract show platelet activating factor antagonistic effects. Kaempferol 3-rutinoside, kaempferol 3-glucoside, kaempferol 3-rhamnosyldiglucoside, quercetin 3-glucoside, quercetin, 2-2hydroxy-1,4-napthaquinone inhibited PAF hypotension. The effect was much stronger than CV-3988 which is a PAF antagonistic agent. In dose greater than 0.01 mg/kg significant inhibition was seen is PAF induced hypotension in mice.

Anti-histamine activity

200 gm of freeze dried white petals of the *Impatiens balsamina* were extracted with 35% cold ethanol for 24 hrs. 8.479 gm residue was obtained after reduction with vacuum. Anti-histaminic activity was estimated using five week old mice. The blood pressure of mice was measured by the tail-cuff method using BP Monitor MK-1000. Each mouse was placed in a holder in the measuring chamber at 37 °C. Mouse was subcutaneously immunized with 50 µg of Hen egg white Lysozyme in incomplete Freund's adjuvant on day 0 to day 9 and the blood pressure was measured. Histamine dihydrochloride in conc. 1, 10 or 1000 mg/kg was dissolved in saline and administered intravenously to normal or HEL sensitized mice. Histamine was considered to exhibit first stage hypotension caused in mice. 100 mg/kg dose of *Impatiens balsamina* was responsible in inhibition of hypotension caused by HEL.

RESULTS AND DISCUSSION

Transcriptional activity

2-methoxy-1,4-napthaquinone was isolated from the 70% ethanolic extract of the aerial part of Impatiens balsamina which

inhibit transcriptional activity (IC₅₀ 2.9 μ M). Luciferase assay was used to estimate the transcriptional activity. Wnt/B-catenin leads to formation of tumors when activated. Cell line STF/293 *i.e.*, human embryonic kidney cell transfected with super TOP Flash were used to determine the decreased transcriptional activity at a concentration >5 μ M (Table 5).

Sr. No	Part	Extract	Activity	Phytoconstituent	Method	Mechanism
1	Leaf	95% ethanolic extract	Antimicrobial	Lawsone Lawsone methyl ether Methylene-3,3'- bilawsone	Disc diffusion assay	Ethanolic extract yielded 2-methoxy- 1,4-napthaquinone which shows activity against gram positive and gram- negative strains in all the fungi.
2	Root and stem	50 mg/kg ethanolic and water extract	Anti-inflammatory	1,4- napthaquinone sodium salts	Carrageenan induced paw edema method bovine serum denaturation assay	<i>I. balsamina</i> roots and stem extract inhibited the lipoxygenase enzyme pathway.
3	Root and stem	0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml hydroethanolic extract	Antidiabetic	Triterpenoids tannins flavonoids anthraquinone	Alpha amylase inhibition assay	Antidiabetic activity is shown by strong inhibition of α- amylase activity.
4	Petals	35% ethanolic extract	Anti-anaphylactic activity	Kaempferol-3- rutinoside	Simulated anaphylaxis in mice	Ethanolic extract inhibited the decreased blood flow .The flower extracts from the plant possesses compounds with weak antihistaminic action and platelet activating factor antagonist activity.
5	Pericarp	35% ethanolic extract	Antidermatic/antipruritic activity	Lawsone kaempferol-3- rutinoside	Atopic dermatitis mice model	The activity of mushroom tyrosinase enzyme was inhibited.
6	Aerial parts	35% ethanolic extract	Anti-androgen activity	Impatienol 3-hydroxy-2-(3- hydroxy-1,4-dioxo (2-napthyl))ethyl naphthalene-1,4- dione	C57BL/6 mice model	Activity of testosterone α- reductase enzyme was reduced.
7	Seed	200 mg/kg Ethanolic extract	Anti-oxidant activity		Reducing power assay, DPPH radical scavenging method and phosphorous molybdenum	Free radical scavenging mechanism was responsible for anti- oxidant activity.

Table 5. Potential applications.

					assay	
8	Whole plant	10%, 25% and 50% hydroalcoholic extract	Anthelminthic activity		Pheretima posthuma model	The observance of non-motility of <i>Pheretima</i> posthuma was an indicator of anthelminthic activity.
9	Leaf	200 mg/kg and 400 mg/kg chloroform and ethanolic extract	Anti-tumor activity	Balsaminone C 2-methoxy-1,4- napthquinone	MTT assay and Dalton's ascites lymphoma tumour bearing mice model.	In-vivo anti-tumour activity by using NIH3T3 and heal cells were performed. It increases the life span and has protective hemopoietic activity. Thus, it shows cytotoxic effects and is used in cancer therapy.
10	Petals	35% ethanolic extract	Anti-histamine activity		HEL and ddY mouse strain using mice tail cuff method	
11	Aerial parts	70% ethanolic extract	Transcriptional activity	2-methoxy-1,4- napthquinone	Luciferase assay	Wnt/B-catenin when activated leads to the formation of tumors. The Wnt/B- catenin pathway was inhibited.
12	Roots, stem, leaf and pods	95% ethanol, ethyl acetate and acetone extract	Anti-H pylori activity	2-methoxy-1,4- napthquinone stigmasta-7,22- diene-3β-ol	Agar dilution method	The zone of inhibition was observed in agar plates due to the inhibition of growth of H-pylori.

Toxicology

Caenorhabditis elegans, a non-mammal was used as an animal model for toxicity studies and biomedical research to study the toxicity. They were used due to their short life span, high sensitivity to the toxins, small body size and ease of handling. The stems of *Impatiens balsamina* are widely used as traditional Chinese medicine to treat a variety of diseases. 35% hydro alcoholic extract of the stems of the plant demonstrated that it widely affected the reproduction, survival, locomotion and life span of *C. elegans*. Lawsone and 2-Methoxy-1,4-Npthaquinone (MNQ) were responsible for the toxicity which was caused by Hydroalcoholic Extracts of *I. Balsamina* Stems (HAEIBS). This was identified using High Speed Counter Current Chromatograph (HSCCC), Nuclear Magnetic Resonance (NMR) and High-Performance Liquid Chromatography (HPLC). 0.2 mg/mL laws one was responsible for reduction in survival rate by 30.5%, reduction in body bends per 20 sec by 36.46% was observed, reduction in head trash per minute by 33.63% and reduction in brood size by 24%. Similar effects were obtained by 10 mg/mL of HAEIBS. 0.2 mg of laws one is similar in action to 10 mg HAEIBS. MNQ is less toxic than laws one.

Another research was conducted on garden balsam to evaluate the acute oral toxicity. The methanol extract of the leaf was fractioned with n-hexane (Liquid-liquid extraction). The screening showed that it contained triterpenoid steroids. 2-3 months old female sprague dawley rats were given the n-hexane fraction and signs of clinical toxicity was observed in the eye, autonomic, somato-motor system, respiratory system and behavioural patterns. At a dose of 5000 mg/KgBw no mortality was caused to rats. This led to the conclusion that it is safe to consume below the dose of 5000 mg/KgBw.

CONCLUSION

Through this updated review the ethnobotanical uses, pharmacological activities as well as toxicity profile of *Impatiens balsamina* L. has been highlighted. The plant possesses wide variety of phytoconstituents with pharmacological activity which will help the researchers for future investigation as well as for formulating novel drugs in pharmaceutical field.

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