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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *IMPATIENS BALSAMINA* SEED (KACI-T-TUMPAI) COLLECTED FROM COIMBATORE DISTRICT, TAMIL NADU, INDIA

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ABSTRACT: The antimicrobial effects of *I. balsamina* seed hexane extract against *Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris*, and *Serratia marcescens* were determined using the standard technique. As well as the phytochemical properties of the extract on the test isolates were also examined using the standard methods. The phytochemical components of the hexane extract of the *I.balsamina* seed include falvonoids, alkaloids, phenol, tannin, alkaloids, steroid, saponin, phenol, flavonoids, triterpenes glycosides and carbohydrate. All the test organisms were susceptible to 25mg/ml of the extract. The MIC and MBC of the hexane extract of the *I.balsamina* against *S. aureus* and *E. coli* was 50mg/ml, while that of *K. pneumonia* and *S. marcescens* were 75mg/ml and 100mg/ml of the extract respectively. The result of this study suggests that the hexane extracts of *I. balsamina* could be suitable for the treatment of diseases infections caused by *S. aureus, K.pneumonia, P. vulgaris and S. marcescens.*

INTRODUCTION: Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [1]. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [2].

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Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity from *I.balsamina* (Family: Balsaminaceae; commonly known as garden balsam) is an annual herb possessing a wide range of medicinal uses [3]. The seeds contain 36–38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition [4]. Although seed extract or oil has been reported to possess antimicrobial activity [5], antioxidant activity [6], antitumor activity and a stimulatory effect on the immune system [7-8], its full potential as an antimicrobial agent has not been exploited. This current study was conducted to investigate the antibacterial activity of the seed extract of *I. balsamina* against pathogenic isolates

of bacteria. The results of this study may further strengthen the recommendation for the use of ethanomedicine in the treatment and control of microbial infections.

MATERIALS AND METHODS: Fresh seed of *I.balsamina* were collected from malasar tribal in Coimbatore, Tamil Nadu India. The collected plant materials were identified in Botanical Survey of India, Coimbatore. The fresh seed of *I. balsamina* seed were allowed to air dry at room temperature for five weeks. The dried seeds were blended into powder and kept in clean air-tight containers for further use.

Extraction procedure of plant materials: About 20g of the powdered seeds was suspended in 200ml of hexane in 500ml sized conical flasks. The extracts in the conical flasks were allowed to infuse for two days at room temperature on a rotary shaker. The hexane extracts was obtained by soxhlet extraction and concentrated by evaporation using a rotary vacuum evaporator [9].

Test organisms: The microorganism employed in the current study were procured from the Kovai Medical Center and Hospital, Coimbatore, Tamil Nadu, India which includes clinical isolates of *P.vulgaris, S. marcescens, S. aureus* and *K.pneumonia*. The bacteria were maintained on nutrient agar slants and stored at 4°C for further studies.

Phytochemical screening of the extracts: A preliminary phytochemical analysis was performed in order to determine the presence of alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids, glycosides, resins, tannins, thiols and peroxidises [10]. The phytochemical study (colour reactions) was performed for the seed extract, using the following procedure.

Test for carbohydrates: To 2 ml of plant extract, 1 ml of Molisch reagent and 4 drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for tannins: To 1 ml of plant extract, 2 ml of 5 % ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins: To 1 ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponin.

Test for flavonoids:

a) To 2 ml of plant extract 1 ml of 1N aqueous NaOH solution was added and observed for the formation of yellow-orange colouration.

b) 2 ml of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange colour.

Test for alkaloids: To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for anthocyanin and betacyanin: To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100ºC. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Test for glycosides: To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride was added. To these 3 drops of concentrated sulphuric acid was added. Presence of greenish blue colour indicates the presence of glycosides.

Test for proteins: To 2 ml of plant extract, 4 drops of 0.2% Ninhydrin was added and heated to 100ºC. Formation of blue colour indicates the presence of proteins.

Test for steroids and phytosterols: To 1 ml of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

Test for phenols: To 1 ml of the extract, 2 ml of distilled water followed by 5 drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

Antibacterial bioassay of crude extracts: Agar-well diffusion technique was used for this purpose. A stock solution of 100mg/ml of the hexane extract was prepared using the extracting solvent and was serially diluted to obtain 50 mg/ml, 25 mg/ml, 15 mg/ml and 5mg/ml. Each labelled medium plate was uniformly inoculated with a standardized inoculums of test organism and a sterile cork borer of 5mm diameter was used to bore wells on the medium into which 0.1ml of the various extract concentration were added. The inoculated plates were kept on the bench for 30mins to allow the extracts to diffuse into the agar medium. The agar plates were incubated at 37^{o}C for 24hours [11]. Antibacterial activities were determined by measuring the diameter of the zones of inhibition (mm) of the extract against the test organisms after incubation.

Minimum Inhibitory Concentration (MIC): The MIC of the potent extracts was determined according to the micro broth dilution technique. Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of seed extracts and incubated at 37°C for 24 hours [12]. The MICs of the hexane extract were read as the least concentration that inhibited the growth of the test organisms.

Minimum Bactericidal Concentration (MBC):
The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was subcultured onto extract-free agar plates, incubated for further 24 hours at 37°C [12]. The concentration at which no growth was observed was taken as the MBCs of the extract against the test organisms.

RESULTS AND DISCUSSION:

Phytochemical screening of the extracts: The phytochemical screening of the hexane extracts of *I. balsamina*. The result indicates the presence of tannin, steroids, saponins, alkaloids, phenol, flavonoids and carbohydrates. Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. The consumption of phytochemicals enhances reduction in the emergence of degenerating diseases. In the present study, the phytochemical analysis of the hexane extract of *I.balsamina* revealed the presence of alkaloids, saponins, flavonoids, phenol, tannins, steroids and glycosides (**Table 1**). The result of this study is in agreement with other previous work [13].

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF DICHLOROMETHANE EXTRACT OF *I. BALSAMINA*

S.no	Phytochemicals	Bioassay
1	Alkaloids	+
2	Glycosides	+
3	Phenol	+
4	Flavonoids	+
5	Saponins	+
6	Steroid	+
7	Tannins	+
8	Triterpenes	+

Key: "+" - positive "-"- negative

The zones of inhibitions (mm) of hexane extracts of *I. balsamina* on *K. pneumonia, P. vulgaris, S. aureus* and *S. marcescens* at concentrations 5mg/ml, 15mg/ml, 25 mg/ml, 500mg/ml and 100mg/ml was represented in **Table 2**. The antibacterial activity of the extract showed that the extract had a broad spectrum of antibacterial activities, inhibiting *K. pneumonia, P. vulgaris, S. marcescens* and *S. aureus* at concentration of ≥50mg/ml. The extract at 25mg/ml was observed to have some activities on *S. marcescens* and *S. aureus* with zone of inhibition 6.0 mm for both. The antimicrobial activity of *I. balsamina* could be due to the abundant presence of phytocompounds which include alkaloids, flavonoids, tannin, saponins and phenol [14]. Flavonoids had shown antibacterial, anti-inflammatory, antiallergic, anti-mutagenic, antiviral, anti-thrombotic and vasodilatory activity [15]. The presence of the flavonoids may have aided the antibacterial activity of the plant.

Tannins on the other hand have astringent properties and hasten the healing of wounds and inflamed mucous membrane while studies have shown that saponins exhibit cytotoxic effect and the growth inhibition against a variety of cell, making them have anti-inflammatory and anticancer properties. They also show tumor inhibiting activity in animals. The presence of tannins and saponins in the present study could be attributed to the use of *I. balsamina* in treating wounds. The presence of phenol in the plant extract further explains the antibacterial properties of the plant as phenols and phenolic compounds have been extensively used in disinfection and remain

the standard with which other bactericides are compared [16].

Antimicrobial activity of the *I. balsamina* extract:

TABLE 2: ANTIMICROBIAL ACTIVITY OF HEXANE EXTRACTS OF *I. BALSAMINA*.

S. no	Test Organism	Concentration of test sample (mg/ml)				
		100	50	25	15	5
1	*Staphylococcus aureus*	18	13	10	6	-
2	*Klebsiella pneumonia*	16	11	9	-	-
3	*Proteus vulgaris*	10	8	4	-	-
4	*Serratia marcecens*	16	14	8	6	-

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the test organisms on the hexane extract of *I. balsamina* was calculated and represented in **Table 3**. The MIC and MBC were 60mg/ml for *P. aeruginosa*, 100mg/ml for *S. marcescens* and 40mg/ml for *P.vulgaris* and *S. aureus* respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aureus* and *E. coli* were 40mg/ml, while for *P. aeruginosa* and *S. marcescens* is 60mg/ml and 100mg/ml respectively. The result of this study showed that *P. aeruginosa E. coli, S. aureus* and *S. marcescens* were susceptible to the hexane extract of *I. balsamina* which is in support of the previous study [17].

TABLE 3: THE MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE HEXANE EXTRACTS OF *I. BALSAMINA*.

S. no	Test Organism	MIC (mg/ml)	MBC (mg/ml)
1	*Staphylococcus aureus*	25	25
2	*Klebsiella pneumonia*	50	50
3	*Proteus vulgaris*	75	75
4	*Serratia marcecens*	100	100

CONCLUSION: The results presented in this report were encouraging, although the clinical studies are needed to know the real efficacy and toxic effects of the plant. The results of phytochemical analysis and antimicrobial studies of the plant extracts confirm their therapeutic use as depicted in the literature. Further detailed analysis of these extracts is required to identify the presence of bioactive compounds responsible for antibacterial and antifungal activities.

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