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ANTIBACTERIAL ACTIVITIES OF VARIOUS SOLVENT EXTRACTS FROM *IMPATIENS BALSAMINA*

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ABSTRACT

The antibacterial activities of Hexane, Petroleum ether, Acetone, Methanol, and Aqueous solution extracts from the plant "*Impatiens balsamina"* was screened against selective bacterial human pathogens namely *Shigella boydii, Salmonella paratyphii, Proteus vulgaris, Staphylococcus aureus, Candida albicans* and *Cryptococcus neoformans* by disc diffusion method. The bacterial pathogens *Shigella boydii, Candida albicans*, and *Cryptrococcus neoformans* showed good positive results (Susceptibility) to the plant extract of "*Impatiens balsamina*" that the zone of inhibition of bacterial pathogens were high in the scale of millimeter ranging from 16 mm to 38 mm. The pathogens *Proteus vulgaris* and *Staphylococcus aureus* shows moderate positive results (Susceptibility) to the plant extracts of "*Impatiens balsamina"* that the zone of inhibition of bacterial pathogens ranging from 1mm to 29 mm. From this above findings, we come to the conclusion that the plant "*Impatiens balsamina"* possesses certain phytochemical properties and these properties in the form of chemicals were responsible for the zone of inhibition formation on the bacterial growth. Therefore, it can be highly recommended for research in the process of drug development for several diseases.

KEYWORDS

Impatiens balsamina, Antimicrobial Activity, human Pathogens, Disc Diffusion Method

INTRODUCTION

Plants have been used as medicinal tools from the earlest days of man's existence. Effective early remedie's were subsequently recorded and documented, leading to the early Herbals. Phamacognosy, the knowledge of drug, grew from these records to provide a disciplined, scientific description of natural materials used in medicine[1]. Herbal remedies are also enjoying a revival as many sufferers turn away from modern drugs and embrace complementary medicine, worldwide most clinical useful prescription drugs are of plant origin[2].

India is considered as the storehouse of medicinal plants. Around 45% of flowering plants are estimated to be having medicinal importance[3]. Also, India is the tenth among the plant rich countries of the world and fourth among the Asian countries[4]. Having time tested traditional system of medicine based on the natural products is the privilege of India.

Development in pharmeceutical industry and chemical techniques replaced crude drugs by synthetic drugs. But, such drugs cause severe adverse reactions. So, even western medicine for eg. Allopathy relies largely on crude drugs, which have stubstances arnalgamated in a fashion, where chemical counter balance the undersitable effects of the other, to give beneficial effects. The advantage of herbal extracts is that the active principles present are diluted and there is only less chance of taking dangerous overdoses[5]. Around 80% of human daily has now turned to community healers and medicinal plants for protection from illness [6]. In spite of tremendous development in the field of modern medicine. Plants still rank in modern as well as traditional medicine throughout the world[7].

From the very earliest days of civilization, mankind has turned to plants for healing. Even today, 80% of the world's population relies upon traditional plant medicine[8]. Medicinal components from plants play an important role in conventional as well as Western medicine plant extracts and compounds derived from plants are in use as drug from the ancient times. In olden days, plants derived from medicines were commonly used in India and China. In western Developed Countries, Plant Constitutes Raw Material For The Preparation Of The Pure chemical derivaties[9].

***Impatiens balsamina* Linn:**

(i) Description:

Annual or Perennial herbs are a little shrubby at the base. Leaves simple, Flowers irregular, axillary, solitary fascicled racemed or umbelled or sometimes scapose. Sepals 3 (or 5), coloured, Imbricate, 2 lateral ones small flat usually green, posterior (lip), the lower in the flower, large, petaloid, often spurred, petals 3 (or 5) the anterior, (Standard) erect, the side ones (wings) entire or 2-3 lobed, Sometimes with a short or long and slender appendage at the base. Stamens 5; anthers cohering, nearly sessile. Disk 0 Ovary oblong, 5-celled, ovules many, 1-seriate in each cell; stigma sessile, 5-toothed. Capsule short (or) long, loculicidally dehiscent; valves 5, elastic. Seeds smooth (or) globose, albumen O. Seeds minutely hirtillous.

(ii) *Distribution*:

In all districts in hilly regions, the wild form of the garden Balsam, are variable in size, leaf and flower; flowers usually pink.

MATERIALS AND METHODS

Antibacterial properties of the flowering plant *Impatiens balsamina* (Geraniaceae) was screened by the method of Kirby-Bauer

sensitivity test using Agar well diffusion method.

(i) Plant Materials:

The study material *Impatiens balsmina* (Geraniaceae) was collected from natural habit (Sholas) of Kolli hills (Namakkal district) in Tamilnadu. This hill station is also a rich reservoir for varieties of medicinal plants and it consists of cool and humid climate.

(ii) Determination Of Antimicrobial Activity: Microbial Strains:

The extracts were tested for the antimicrobial activity. The microbial strains employed in the biological assays were *Shigella boydi, Salmonella paratyphii, Proteus vulgaris, Staphylococcus aureus, Candida albicans,Cryptococcus neoformans* obtained from Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh.

(iii) Determination Of Antibacterial Activity By Kirby - Bauer Sensitivity Test:

To become acquainted with the Kirbly-Bauer procedure for the evaluation of the antimicrobial activity of the plant extract.The medium of choice is Mueller-Hinton Agar (MHA) for bacteria, Sabouraud Dextrose Agar (SDA) for fungus, with a pH of 7.2 to 7.4, which is poured into plates to a uniform depth of 5mm and refrigerated on solidification. Prior to use, the plates are transferred to an incubator at 37oC for 10 to 20 minutes to dry off the moisture that develops on the agar surface. The plates are then heavily inoculated with a standardized inoculum by means of a cotton swab to ensure the confluent growth of the organism. The discs are aseptically applied to the surface of the agar plate at well spaced intervals. Once applied, each disc is gently touched with a sterile applicator stick to ensure its firm contact with the agar surface. Do not press the discs into the agar medium.

Following incubation, the plates are examined for the presence of growth inhibition, which is indicated by a clear zone surrounding each disc. The susceptibility of an organism to a plant extract is determined by the size of this zone, which itself is dependent on variables such as:

1. The ability and rate of diffusion of the plant extract into the medium and its interaction with the test organism.
2. The number of organisms inoculated.
3. The growth rate of the organism.
4. The degree of susceptibility of the organism to the plant extract.

Based on the inhibition zone in diameter, the test organism is determined to be resistant, intermediate or susceptible to particular plant extract.

RESULTS

The antibacterial activities of Hexane, Acetone, Methanol, Petroleum ether and Aqueous solution extracts from the plant "*Impatiens balsamina"* was screened against selective microbial pathogens namely *Shigella boydii, Salmonella paratyphii, Proteus vulgaris, Staphylococcus aureus, Cryptococcus neoformans* and *Candida albicans* by disc diffusion method (Table 1)

Table: 1
Antimicrobial activity of Impatiens balsamina

Microorganisms	Hexane				Aqueous				Acetone				Methanol				Petroleum ether			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
Bacterial pathogen																				
Shigella boydii	16	16	21	21	0	0	0	0.3	19	2	21	22	2	21	24	25	21	21	25	26
Salmonella paratyphii	0	0.9	1	0.6	0	0	0	0	0	1	0	0.9	0	0	0	0	0	0.2	0	0
Proteus vulgaris	0.9	0.9	1	1	0	0	0	0	0	1	19	14	0	1	13	12	1.3	1.4	1.6	1.2
Staphylococcus aureus	18	19	24	24	15	0	15	0	19	24	25	23	2	22	25	23	21	25	29	24
Cryptococcus neoformans	14	15	17	28	13	0	0	0	18	21	2	21	16	2	29	28	18	13	22	21
fungal pathogen																				
Candida albicans	26	29	29	25	0	0	0	0	27	3	33	38	25	31	29	35	3	38	34	37

(i) Antimicrobial activity of Impatiens balsamina:

The antimicrobial activity of Hexane, Acetone, Methanol, Petroleum ether and Aqueous solution extracts of *Impatiens balsamina* was presented in Table -1

The results from the Hexane extract reveal that maximum zone of microbial growth inhibition was recorded in *Candia albicans* (29 mm in 50 μl, 75 μl), *Staphylococcus aureus* shows 24mm in 75 μl and 100 μl and 19 mm in 50 μl, and 18 mm in 25 μl), *Shigella boydii* shows (21 mm in both 100 μl and 75 μl and 16 mm in both 25 μl and 50 μl) *Cryptococcus neoformans* shows growth inhibition zone of 28 mm in 100 μl, 17mm in 75 μl, 15 mm in 50 μl and 14 mm in 25 μl volume. Moderate activity in *Salmonella paratyphi* with 0.9 mm (50 μl), 0.6 mm (100 μl) 1mm (75 μl) and 0.9mm (25 μl), 0.9m (5 μl), 1 mm (75 μl) and 1 mm (100 μl) inhibition recorded in the Proteus vulgaris. The results from the Acetone extract reveal that maximum zone of microbial growth inhibition was recorded in *Candida albicans* (38 mm in 100 μl, 33 mm in 75 μl, 27 mm in 25 μl and 3 mm in 50 μl).

Staphylococcus aureus shows that maximum zone of inhibition also recorded in the Acetone extract that 25 mm (in 75 μl), 24 mm (50 μl) 23 mm (100 μl) and 19 mm (25 μl). *Cryptococcus neoformans* shows that maximum zone of inhibition was recorded in the 21 mm (50 μl and 100 μl), 18 mm in 25 μl and 2 mm (75 μl).

Shigella boydii showed that maximum zone of inhibition was recorded in 22 mm in 100 μl, 21 mm in 75 μl, 19 mm in 25 μl and 2 mm in 50 μl. Moderate growth inhibition activity was recorded in *Salmonella paratyphii* with 0.9 mm in 100 μl, 1 mm in 50 μl and no growth inhibition zone in the 75 μl and 25 μl of Acetone extract. *Proteus vulgaris* pathogen shows moderate growth inhibition zone in the Acetone extract with 14 mm in 100 μl, 1 mm in 50 μl, 19 mm in 75 μl, and no zone in the 25 μl. The antibacterial activity of methanol extract shows 25mm in 100 μl, 24 mm in 75 μl, 21 mm in 50 μl, and 2 mm in 25 μl in the *Shigella boydii* which shows the maximum zone of inhibition. *Candida albicans* shows zone of inhibition with 35 mm in 100 μl, 31 mm in 50 μl, 29 mm in 75 μl, and 25 mm in 25 μl. *Cryptococcus neoformans* shows zone

of inhibition in 29 mm in 75 µl, 28 mm in 100 µl, 16 mm in 25 µl, and 2 mm in 50 µl. Moderate activity of growth inhibition was recorded in *Salmonella paratyphii* that 1 mm in 100 µl and all other results are clear that no zone is formed. *Proteus vulgaris* shows that 13 mm in 75 µl, 12 mm in 100 µl, 1 mm in 50 µl and no zone also recorded in the 25 µl. *Staphylococcus aureus* shows zone of inhibition in 25 mm in 75 µl, 23 mm in 100 µl, 22 mm in 50 µl and 2 mm in 25 µl. The results from the Petroleum other extract reveals that maximum zone of microbial growth inhibition was recorded in *Candida albicans* with 38 mm in 50 µl, 37 mm in 100 µl, 34 mm in 75 µl and 3 mm in 25 µl. *Staphyloccocus aureus* shows maximum inhibition zone of microbial growth that 29 mm in 75 µl, 25 mm in 50 µl, 24 mm in 100 µl and 21 mm in 25 µl. *Cryptococcus neoformans* reveals that maximum inhibition zone was recorded in 22mm in 75 µl, 21 mm in 100 µl, 18 mm in 25 µl, and 13 mm in 50 µl. The results from the *Shigella boydii* in the Petroleum ether extract shows that 26 mm in 100 µl, 25 mm in 75 µl, 21 mm in 50 µl, and 21 mm in 25 µl. The results from the Petroleum ether extract in respect of *Salmonella paratyphii* shows that moderate inhibition activity with 1 mm in 100 µl, 0.2 mm in 50 µl and no zone activity was found in the 75 µl and 25 µl. *Proteus vulgaris* pathogen shows that moderate growth inhibitions zone has recorded as 1.6 mm in 75 µl, 1.4 mm in 50 µl, 1.3 mm in 25 µl and 1.2 mm in 100 µl. Aqueous solution extract for all the bacterial pathogens namely *Shigella boydii, Salmonella paratyphii, Proteus vulgaris, Staphylococcus aureus, Candida albicans*, and *Cryptococcus neoformans* shows no growth zone formation since the aqueous solution extract contains no medicinal properties.

DISCUSSION

There has been so for no study found, on the Antibacterial activities of "*Impatiens balsamina".* To fill this lacuna, the present investigation was taken up and carried out.

The 4 species, *Candida albicans* showed maximum zone of inhibition (38 mm, 34 mm, and 37 mm) in the Petroleum ether extract, followed by Acetone extract (38 mm, 33 mm and 27 mm) Methanol extract (35 mm, 31 mm, 29 mm and 25 mm) and (29 mm, 26 mm and 25 mm) in the Hexane extract.

In case of *Cryptococcus eoformans*, maximum zone of inhibition was observed in the Methanol extract (29mm, 28 mm, 16 mm) followed by Petroleum ether extract (22 mm, 21 mm, 18 mm, 13 mm) and Hexane extract (28mm, 17 mm, 15 mm and 14 mm). In case of *Staphylococcus aureus* maximum zone of inhibition was observed in the Petroleum ether extract (29 mm, 25 mm, 24 mm and 21 mm) Methanol extract (25 mm, 23 mm and 22 mm), Acetone extract (25 mm, 24 mm, 23 mm and 19 mm) followed by Hexane extract (24 mm, 19 mm and 18 mm).

The pathogen *Shigella boydii* showed maximum zone of inhibition in the Petroleum ether extract (26 mm, 25 mm and 21 mm), Methanol extract (25 mm, 24 mm and 21 mm), Acetone extract (22mm, 21 mm and 19 mm) followed by Hexane extract (21 mm, 16 mm).

Another two pathogens *Salmonella paratyphii* and *Proteus vulgaris* showed moderate zone of inhibition in all the four extracts namely Hexane, Acetone, Methanol, Petroleum ether from the range of 19 mm to 0.2 mm.

Ultimately, the control discs with Aqueous solution were maintained in all the petriplates. On examination, when the other plant extracts showing growth inhibition of bacterial pathogens, the control discs did not shows any positive result.

From this, it was obvious that the plant extract of "*Impatiens balsamina*" possesses unique phytochemical properties and these chemicals were responsible for the zone of inhibition on the bacterial growth.

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