

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *DATURA METEL* AND *VITEX NEGUNDO* AGAINST BACTERIAL COLD WATER DISEASE CAUSING ORGANISM

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ABSTRACT

Objective: Effect of medicinal plants on pathogens and disease causing organism are believed to possess good activity against it. The medicinal plant *Vitex negundo* and *Datura metel* were studied for analyzing antibacterial effect against Bacterial Cold Water Disease causing organism.

Methods: The selected plant leaves were extracted out with following solvent of Hexane Chloroform and Aqueous and tested against *Flavobacterium psychrophilum*. Paper Disc diffusion method was followed with varying concentration of 50-250 mg/ml and then these plants were subjected to preliminary phytochemical analysis by following standard protocol of Harbone's phytochemical analysis.

Results: The phytochemical analysis resulted in presence of terpenoids, phytosterols, steroids, flavonoid and phenol compounds. Some of the secondary metabolites that could have acted against *Flavobacterium psychrophilum* for its sensitivity against plant extracts, pathogen showed antibacterial activity of 15mm sensitivity at 250 mg/ml concentration for both plant extracts and sensitivity were seen from 150mg/ml concentration.

Conclusion: The medicinal value rich plants could be used in treating or preventing Bacterial Cold Water disease or infection with higher specification of its bioactive compound for the development of chemical constituent or drug molecules.

Keywords: medicinal plants, *Flavobacterium psychrophilum*, paper disc diffusion, sensitivity, and phytochemicals.

INTRODUCTION

Plants used in traditional medicine contain a vast variety of compounds and those compounds are used to treat chronic and infectious diseases. Moreover, it is the oldest form of health care known to humankind and it lacks documentation in the lights of modern medicine. WHO gave a report that more than 80% of world's populations depends on traditional medicine for their primary healthcare needs [1]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. A natural product from higher plants gives a new source of antimicrobial agents with possible higher novel mechanism of action against pathogens. These agents are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. *Vitex negundo* Linn [Verbenaceae] consists of 250 species of which 14 species found in India. Its vernacular name is Nirkudi/Nallanocci/Nochi. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches [2]. Bark is thin and yellowish grey; leaves are foliolate, leaflets lanceolate, lateral are smaller; upper surface of the leaves is green and lower surface is silvery in color. Flowers are bluish purple, black when ripe, whereas roots are cylindrical, long woody, and tortuous with grey brown color [3]. Roots are tonic, febrifuge, antirheumatic, diuretic, expectorant and used as a demulcent in dysentery, in cephalalgia, otalgia, colic, uropathy wound and ulcers. Bark is useful in odontalgia, verminosis and ophthalmopathy. Leaves are aromatic, bitter, acrid, astringent, anodyne, anti-inflammatory, antipyretic, bronchial smooth muscle relaxant, antiarthritic and antihelminthic. Flowers are cool, astringent, carminative, hepatoprotective, digestive, febrifuge and useful in cardiac disorders. Fruits are nervine, cephalic, aphrodisiac, emmenagogue and vermifuge [4].

Datura metel Linn also called as Indian Thorn Apple. The family solanaceae consists of 85 genera and 2500 species worldwide. *Datura metel* Linn is a perennial herbaceous plant and can reach height of 1.5m. Leaves are simple alternate, dark green, broadly

ovate, shallowly lobed and glabrous. Flowers are large, solitary and trumpet-shaped with a sweet fragrance. The fruit is in the form of capsule with short spine covering. The name *Datura* comes from early Sanskrit "Dustura or Dahatura" [5]. Indian thorn apples flowers often-depicted in Hindu Tantric art, usually in connection with incarnations of Shiva. *Datura metel* remains a psychoactive plant of great ethnopharmacological significance especially in India, Southeast Asia and Africa. In traditional Chinese medicine, it is used to treat asthma, chronic bronchitis, seizures and coma. The plant finds application in the treatment of catarrh, epilepsy, hemorrhoids, painful menstruation, skin ulcers and wounds. It is used to treat laryngitis and treacheries [6]. The plant has been used to treat impotence, asthma, diarrhea as an analgesic to control fever and as a drug for criminal purposes [7][8].

Bacterial Cold Water Disease

It was first described by Borg in 1948 and the causative organism was identified as *Flavobacterium psychrophilum* which produces acute septicemic infection in salmonids and few other species [9][10]. *Flavobacterium psychrophilum* is a Gram-negative bacterium that occurs in low temperatures and a clinical sign on the fish exhibits large open lesions on the tail region, necrosis may progress deep to the muscle tissue and erode vertebrae and erosion of fins. The most affected are younger fishes, which has greater severity of the disease. Cold-water disease also reported in Atlantic salmon, Chinook salmon, Brook trout and Brown trout [11][12][13]. The commercial trout industry of the United states experienced severity of the disease in younger fishes at 15°C and *Flavobacterium psychrophilum* has affinity for skin and muscle tissue and the onset of feeding lesions with yellow colored edges may form on the caudal peduncle region [13][14]. In heavily infected fish, large number of the bacteria can be seen in the liver, spleen, air bladder, pancreas and heart that indicate the septicemic nature of the disease [9]. The transmission of the bacteria takes place by the surface contact of the fish to another which results in causing the infection and forming the lesion or damage to the skin [15]. Preventing the causative agent was

difficult due to ubiquitous nature of the microorganism and the trout farms were using stress management and minimizing the damage to the skin, but the chemical compound usage were limited due to the unique nature of the *Flavobacterium psychrophilum*. There are no approved drugs to treat cold-water disease however, antimicrobial test susceptibility were assed with ampicillin, amoxicillin-clavulanic acid. Enrofloxacin, florfenicol, gentamicin, ciprofloxacin and sulfamethoxazole-trimethoprim and resulted in maximum of resistance and sensitive to enrofloxacin against it [16],

MATERIALS AND METHODS

Plant collection

The medicinal plants were collected from the nearby field with help of people with knowledge about the plant.

Preparation of the extract

The leaves of *Vitex negundo* and *Datura metel* were washed with distilled water and shadow dried for 3-5 weeks. Then it was cut into small pieces, powdered with electric blender and stored for future use at 4°C,

Extraction

The plant extraction was carried out by Soxhlet apparatus for 24 hrs. The powdered sample was added to sohxlet apparatus and solvent is added to it in 1:5 ratios. The solvent system used in as increasing order of polarity [Hexane-Chloroform-Distilled water]. Then the extract was collected from sohxlet apparatus and kept in room temperature for air-drying, The residues were collected, weighed and stored in 4°C for future use,

Concentration of the extract

The residues were weighed and dissolved in its mother solvent at 1% W/V concentration,

Composition of Wakimoto Medium

Ingredients	gm/lit
Ca(NO ₃).4H ₂ O	0.5
Na ₂ HPO ₄ .12H ₂ O	2.0
Peptone	5.0
Sucrose	15.0
FeSO ₄ .7H ₂ O	0.5
Agar	15.0

Composition of Nutrient agar

Ingredients	gm/lit
Beef extract	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0
Agar	15.0

Composition of Nutrient broth

Ingredients	gm/lit
Beef extract	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0

Bacterial species

The fish pathogen *Flavobacterium psychrophilum* MTCC NO: 2495 has been procured from Microbial type culture collection centre, IMTECH, Chandigarh, India. The bacterial culture of *Flavobacterium psychrophilum* was sub cultured in the modified Wakimoto medium slants at 30°C and stored at 4°C for future use. For the antibacterial screening of the organism, it was sub cultured in the nutrient broth and tested in nutrient agar at 30°C.

Antibacterial assay

Paper disc diffusion method

Paper disc diffusion method was followed to study the effect of extract against bacterial pathogens. Paper disc was prepared by cutting 6mm of sterilized Whatman No.1 filter paper, the disc were loaded with varying concentration of the extract and incubated, and it was sub cultured in the nutrient broth and swabbed in the Petri plates with media and kept for incubation at 37°C for 24hrs to 48 hrs.

Phytochemical analysis

The phytochemical analysis techniques were followed for solvent extracts of *Vitex negundo* and *Datura metel* using standard protocol [17]. The plant species above described were used for treating disease in natural therapeutics and in the form of chemical compounds but it was not used to treat this type of infectious disease which cause in the salmonid fishes.

Antibacterial effect over *flavobacterium psychrophilum*

The extracts of *Vitex negundo* and *Datura metel* leaves were tested against *Flavobacterium psychrophilum* and the formation of the zone was observed for the five different concentrations.

Media components

The media compounds were purchased from Micro fine chemicals

Table1: Phytochemical analysis of *Vitex negundo* extracts.

Compounds	Hexane extract	Chloroform extract	Aqueous extract
Alkaloid	-	-	-
Flavonid	+	+	+
Phenols	-	+	+
Terpenoids	+	+	-
Glycosides	-	-	+
Saponins	-	-	+
Steroids	+	+	-
Volatility	+	+	+

+ Presence; - absence

Table 2: Phytochemical analysis of *Datura metel* extracts

Compounds	Hexane extract	Chloroform extract	Aqueous extract
Alkaloid	-	+	-
Flavonid	+	+	+
Phenols	-	-	+
Terpenoids	+	+	+
Glycosides	-	-	-
Saponins	-	-	+
Steroids	+	+	+
Phytosterol	+	+	-
Volatility	+	+	+

+ Presence; - absence,

Vitex negundo extracts

S. No.	Concentration of the disc mg/ml	Hexane extract	Chloroform extract	Aqueous extract
1	50	-	7mm	-
2	100	-	9mm	-
3	150	7mm	10mm	9mm
4	200	9mm	12mm	11mm
5	250	15mm	15mm	15mm

Datura metel extracts

S. No.	Concentration of the disc mg/ml	Hexane extract	Chloroform extract	Aqueous extract
1	50	-	-	-
2	100	-	6mm	-
3	150	-	9mm	7mm
4	200	-	12mm	9mm
5	250	7mm	15mm	11mm

RESULTS

Phytochemical analysis

The extracts of the plant extracts has given the presence of their secondary metabolites

DISCUSSION

The extract of Nochi leaves [*Vitex negundo*] exhibits 15mm of inhibition zone for the test extracts at 250mg/ml. Moreover, chloroform extracts were good from the concentration of 150mg/ml of the disc concentration to the maximum concentration tested. For the extracts of *Datura metel* leaves the high zone of inhibition was observed at 15mm at the chloroform extract at 250mg/ml concentration and 11mm of inhibition zone was observed at 250mg/ml of the disc concentration. *Agele marmelos* bark extracts also studied for its antibacterial activity against *Flavobacterium psychrophilum* and plant extract that showed sensitivity level of 15mm and comparatively studied with commercial antibiotic [18]. The phytochemical analysis of the *Vitex negundo* and *Datura metel* leaves shows the presence of terpenoids, phytosterol and steroid in the hexane extracts, Phenols, volatile oils in the chloroform extracts and Flavonid, glycosides saponins in the aqueous extracts of the plant respectively.

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