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PRELIMINARY PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL EVALUATION OF *VIBURNUM PUNCTATUM* BUCH - HAM. EX D. DON

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Keywords:

Viburnum punctatum leaves, Ethanol, Ethyl acetate, Acetone, Phytochemical screening, Antimicrobial activity

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ABSTRACT

Viburnum punctatum Buch.-Ham. ex D. Don belongs to Caprifoliaceae family is a medicinally important plant also known as “Konakaram” commonly in Tamil. It is a small evergreen tree, commonly found in moist forests and in sholas, above 1200m in South east Asia.Many species of *Viburnum* are recognized for their medicinal properties from very early times of this century. The *Viburnum punctatum* leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti periodic effect. In present study, the phytochemical investigation was carried out with different organic solvents like ethanol, ethyl acetate, acetone and aqueous extract of *Viburnum punctatum* leaves (Caprifoliaceae). The preliminary phytochemical studies exhibited the presence of alkaloids, flavonoids, carbohydrates, glycosides, phenolic compounds and tannins, proteins and aminoacids, saponins, steroids and terpenoids, quinones and coumarins as chemical class present in the extracts. The result showed the ethanol extract appeared to be the most effective and more potent against those obtained with other solvents. The ethanol extract of *V.punctatum* were most active, showed higher activity against *Staphylococcus aureus* when compared to other bacterial strains which can be attributed due to the presence of these phytochemicals.

INTRODUCTION

India, our country because of its diverse agroclimatic conditions has a substantial collection of medicinal plants and herbs. But only a small proportion of the natural plant sources are tapped scientifically (Kirtikar, Basu, 1987). Nilgiris is known as a are not seen in any other part of the country. Out of the described plant species in "The flora of Nilgiris and Pulney hill tops", more than 10% are known only from the Nilgiris (Fyson, 1974).

An increasing number of investigator's have been devoting attention to the vast store of knowledge of plant properties and uses still intact in native culture in several parts of the world. And not withstanding the rapid acculturation and consequent loss of plant lore in many areas, there remains much to be done before this abundance of knowledge shall be for ever entombed with the culture that gave it birth (Jain, 1991).One cannot assure that all of these plants possess a long recorded history, although they have been also reported to contain medicinally valuable phytochemical-pharmaceuticals and are subjected to formulate siddha, ayurvedic, unani and Chinese system of medicine (Kathiresan Prabhu *et al*, 2011).

According to the analysis by WHO (World Health Organization), nearly 80% of the world population were depending on herbal medicines for their health care problems (Farnsworth *et al*., 1985). The plants' medicinal use actually due to the presence of some active components that are effective for human body in many ways (Akinmoladun *et al*., 2007). Flavonoids, terpenoids, alkaloids, essential oils, tannins, saponins and phenolic compounds are some of the chemical constituents responsible for bioactivity of plants (Edeoga *et al*., 2005; Tan *et al*., 2006). Synthesis of these compounds are responsible for the plants ability to resist themselves from predators as well as to destroy pathogenic microorganisms (Mans *et al*., 2000;Yamin Bibi *et al*, 2010). Medicinal plants and microorganisms are the rich sources of secondary metabolites that are the potential sources of useful drugs and other useful bioactive products (Dung N.X *et al*., 1991, Alagesaboopathi C *et al*. 2011).

Viburnum punctatum Buch.-Ham.ex D.Don (*Viburnum acuminatum* Wall) belonging to Caprifoliaceae family, is a medicinal plant under the order Dipsacales (Prabhu *et al*., 2009). It belongs to the monotypic genus *Viburnum*, native to India, Nepal, Bhutan, Thailand, Cambodia, Vietnam, Indonesia and China. It is a shrub or medium sized tree, growing at an altitude not less than 1500 m; profusely with other plants in Nilgiri, Himalaya and Coimbatore. The leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti-periodic effect.

MATERIALS AND METHODS

Plant material collection

Viburnum punctatum leaves were collected from The Nilgiri Hills, Tamil Nadu, India.

Organic solvent extraction Freshly collected leaves of *Viburnum punctatum* were shade dried and then powdered using a mechanical grinder. 10 grams of pulverized leaf material were soaked separately in 250 ml ethanol, ethyl acetate, acetone and kept on a rotary shaker for 24 hours. The extracted material from solvents was filtered through a Whatman No. 1 Filter Paper in separate flasks and the process was repeated until all the soluble compounds had been extracted. The extract was then concentrated under reduced pressure in a rotary evaporator, weighed and stored at 4°C until further analysis.

Preparation of dilution The dried ethanol, ethyl acetate, acetone and aqueous extracts were finally dissolved in their respective solvents in a proportion of 10 mg/ml.

Phytochemical Analysis

The preliminary phytochemical analysis was carried out on plant leaf extracts using standard procedures (5-8) to identify the phytochemical constituents. They are:

Detection of Alkaloids (Mayer's test): 0.5g of extract dissolved in 5 ml of 2N HCL and filtered. Then the filtrate were treated with Mayer's reagent (Potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of Alkaloids.

Test for Carbohydrates (Fehling's test): Equal volume of Fehling's A and Fehling's B reagent mixed together, 2ml of it was added to the extract and boiled gently. A brick red precipitate was appeared at the bottom of the test tube indicates the presence of reducing sugars.

Test for Cardiac glycosides: 5 ml of the extract is treated with 2 ml of glacial acetic acid containing 1-2 drops of 2% ferric chloride solution. This mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring of the interface indicates the presence of cardiac glycosides.

Test for Flavonoids: 0.5 ml of the extract treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which turns colourless on addition of dilute acid, indicates the presence of Flavonoids.

Test for Glycosides: 0.5g of the leaf extract was stirred with 10ml of boiling distilled water. This was then filtered and 2ml of that filtrate hydrolyzed with a few drops of concentrated HCL and the solution was rendered alkaline with a few drops of ammonia solution. 5 drops of the solution were added to 2ml of Benedict`s qualitative reagent and boiled. The appearance of reddish brown precipitate showed the presence of Glycosides.

Test for Phenols: 0.5 ml of leaf extract treated with 3-4 drops of ferric chloride solution. The formation of bluish black colour indicates the presence of Phenols.

Test for Proteins (Millon's test): A small portion of extract when mixed with 2ml of Millon's reagent, White precipitate appeared which turn red upon gentle heating confirmed the presence of Protein.

Test for Saponins (Frothing Test): The extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The persistance of frothing indicates the presence of Saponins.

Test for Steroids: The extract was mixed with 2ml of chloroform and the concentrated sulphuric acid is added sidewise. A red colour was produced in the lower chloroform layer indicated the presence of Steroids.

Test for Terpenoids: 5 ml of leaf extract was mixed in 2ml of chloroform, and the concentrated sulphuric acid (3 ml) was carefully added to form a layer. The reddish brown colouration in the interface formed to show positive result for the presence of Terpenoids.

Test for Tannins: The leaf extract was mixed with 2 ml of 1% Ferric chloride solution. A blue-black precipitate formation indicates the presence of Tannin.

Detection of Gums and Mucilages: About 10 ml of aqueous extract was added to 25ml of absolute alcohol with constant stirring. The precipitate was then dried in air and examined for its swelling property.

Test for quinones: 2 ml filtered extract mixed with 2 ml of concentrated sulphuric acid. The formation of red color indicate the presence of quinones.

Test for coumarins: 2ml filtered extract mixed with 2 ml of 10% Sodium hydroxide solution. The formation of yellow color indicate the presence of coumarins.

Standard chemical tests were performed for Qualitative analysis of different fractions and the results were tabulated.

Antibacterial activity Test:

The selected microorganisms (*Staphylococcus aureus, Bacillus subtilis, Enterococcus sp, Escherichia coli, Klebsiella pneumonia and Proteus mirabilis*) were collected from stock culture and kept on Muller- Hinton agar. The antibacterial activity was carried out on standard agar well - diffusion method (Perez et al., 2009). The pure cultures of bacterial pathogens were sub cultured on nutrient broth. 100µl of fresh overnight grown cultures of the bacteria were spread on Muller Hinton Agar plates and each strain was spread uniformly using L - rod. 0.5ml of plant extract were potted. Kanamycin (5µg / ml) was used as standard, inoculation done for one hour to make

the possible diffusion of antibacterial agent into the medium. This inoculated plates were then incubated at 37°C for 24 hours and the diameter of zone of inhibition for bacterial growth was measured in millimeter in plates (Eryilmaz M *et al*., 2013).

Statistical Analysis

All the values of activity were expressed as the means ± SD of three samples. The data were analysed using ANOVA technique and the means were compared using least significance difference (LSD) at 5% (0.005) probability level. (Steel and Torrie, 1980).

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOL, ETHYL ACETATE, ACETONE AND AQUEOUS EXTRACTS

Test	Ethanol Extract	Ethyl acetate Extract	Acetone Extract	Aqueous Extract
Alkaloids	+	-	+	+
Carbohydrates	+	-	+	+
Cardiac glycosides	-	-	-	-
Flavonoids	+	-	+	+
Glycosides	+	-	-	-
Phenols	+	+	+	+
Proteins & aminoacids	-	-	-	+
Saponins	+	-	+	-
Steroids	-	-	+	-
Terpenoids	+	+	-	+
Tannins	+	-	-	+
Gums & Mucilages	-	-	-	-
Quinones	+	+	-	+
Coumarins	+	+	+	+

+ indicate present - indicate absent

TABLE 2: ANTIBACTERIAL SENSITIVITY ACTIVITY OF *V.PUNCTATUM* ETHANOLIC LEAF FRACTIONS

S.No	Microorganism	Gram	Antibiotic (Kanamycin (5 µg/ ml)	Mean diameter of zones of inhibition	P value
1	*Staphylococcus aureus*	Positive	2.96 ± 0.06	1.10 ± 0.10	0.008
2	*Bacillus subtilis*	Positive	2.43 ± 0.45	0.73 ± 0.06	0.001
3	*Enterococcus sp*	Positive	2.00 ± 0.00	0.93 ± 0.12	0.015
4	*Klebsiella pneumoniae*	Negative	2.87 ± 0.06	0.87 ± 0.06	0.026
5	*Escherichia coli*	Negative	2.40 ± 0.10	1.00 ± 0.10	0.031
6	*Proteus mirabilis*	Negative	2.37 ± 0.12	1.10 ± 0.10	0.020

Values are the mean ± SD of three samples

RESULT AND DISCUSSION

The results of the phytochemical investigation carried out on different organic extracts, were the preliminary photochemical screening revealed the presence of flavonoids, alkaloids, carbohydrates, glycosides, phenolic compounds and tannins, proteins and aminoacids, steroids and terpenoids, saponins, quinones and coumarins. The presence of active constitutents were

found more in ethanolic extract when compared to other organic solvents. The proximate analysis was also carried out to identify the purity of the materials.It was concluded that the ethanol extract of *Viburnum punctatum* leaves contain more important constitutents for pharmacological activity. The plant also reported to contain triterpenes, saponins in root, mucilage, tannin and lignin in leaf, saponins, starch grains and tannins in stem and glycoside, terpenoid and sterols in leaves (Renjith Alex *et al*., 2014). The folkloric information alongside also claims about the root of Viburnum species that the plant root may have antimicrobial, antiinflammatory, antidiabetic, antioxidant, cytotoxicity, antiulcer and antispasmodic properties due to the presence of triterpenoids, phenolic compounds, flavonoids, saponins, tannins and anthocyanins (Kathiresan Prabhu *et al*., 2009 and 2011). The presence of these phytochemical constituents will show the reason behind the antimicrobial property of the plant extracts, since all these secondary metabolites has been already reported for their antimicrobial property which can be used in the treatment of infectious diseases caused by common human bacterial pathogens (Zahid Iqbal Awan et al., 2013).

The biological activity of these bioactive fractions were confirmed through antibacterial assay. The assay revealed highest activity against *Staphylococcus aureus* (1.10 ± 0.10mm) and other bacterial strains like *Bacillus subtilis* (0.73 ± 0.06mm), *Enterococcus sp* (0.93 ± 0.12mm), *Escherichia coli* (1.00 ± 0.10mm), *Klebsiella pneumonia* (0.87 ± 0.06mm) and *Proteus mirabilis* (1.10 ± 0.10mm). The extract possess bioactive chemical constituents explore antibacterial properties (Ghias uddin et al., 2013). Renjith Alex et al (2014) reported that the medicinal plant *V.punctatum* used in Indian- herbal medicine also exhibit good anti fungal activity against *Candida albicans* in methanolic extract.Thus these findings can form the basis for further studies to isolate active compounds, elucidate structures, toxicity testing and further investigation for their applications in pharmaceutial purposes Yamin Bibi *et al*., 2010).

CONCLUSION

The present evaluation revealed the screening of qualitative analysis and antibacterial assay on organic solvent extracts of leaves of *V.punctatum* and exhibited best antibacterial activity against six selected bacterial strains with better inhibition zone which can be used in the treatment of infectious diseases caused by common human bacterial pathogens.The study of the following ethanolic extract can also be selected for the free radical scavenging activity in adjuvant induced arthritic rats by determining the Lipid peroxidation in liver and plasma.

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