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Research Article

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Phytochemical screening and antimicrobial activity of extracts of *Viburnum punctatum* Buch-Ham Ex D. Don against selected microbes

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ABTRACT

The Phytochemical investigation was carried out on the crude chloroform and methanol extracts of the aerial parts of Viburnum punctatum (Caprifoliaceae). The antimicrobial activity of the extract was tested against chemical isolates of some bacteria using the disc diffusion method. The preliminary phytochemical studies revealed the presence of alkaloids, glycosides, flavonoids, fixed oils and fats, phenolic compounds and tannins, proteins and aminoacids, gums and mucilage, phytosterols and saponins as chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of the gram positive bacteria such as Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus, gram negative bactria is Escherichia coli and Candida albicans. The results showed that the methanol extract was more potent than the chloroform extract.

Key words: *Viburnum punctatum*, Antimicrobial activity, Phytochemical screening.

INTRODUCTION

Antimicrobial chemotherapy has been an important medical treatment since the first investigations of antibacterial dyes by Ehrlich in the beginning of the 20th century. Bacterial resistance forces the research community to develop methods of altering structures of antimicrobial compounds to avoid their inactivation, yet structural modifications alone are not enough to avert bacterial resistance. The increasing use of household antibacterial products and agricultural antimicrobials fosters resistance to drugs specific for human therapy acid may have huge consequences for particularly children and elderly [1] [2] [3].

Antimicrobials are used worldwide in human medicine, food, agriculture, livestock and household products. In many cases the use of antibiotics is unnecessary or questionable. Consumption of antibiotics is linked to bacterial resistance. In hospitals, most common resistant bacteria include methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant enterococci and gram negative rods, including the *Enterobacteriaceae* and *Pseudomonas aerugnosa* [4].

Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with antimicrobial activity with possibly new mode of action. This expectation that some naturally occurring plant compounds can kill anti-biotic-resistant strains of bacteria such *as Bacillus cereus, Escherichia coli, Micrococcus lutens* and *Staphylococcus aureus* has been confirmed by friedman *et. al* (2006). A pubmed search for the antimicrobial activity of medicinal plants produced 115 articles from the period between 1966 and 1994. However in the following decade between 1995 and 2004, this number more than doubled to 307. In these studies one finds a wide range of criteria related to the discovery of antimicrobial compounds in plants. Many focus on

determining the antimicrobial activity for plant extracts found in folk medicine or its isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones diterpenes, triterpenes or napthoquinones [5].

Viburnum punctatum (*Viburnum acuminatum* Wall) family Caprifoliaceae. It is shrub or small trees, evergreen, to 9mm tall. It belongs to monotypic genus *Viburnum*, native to India, Indonesia, Bhutan, Cambodia, Nepal, Thailand, Vietnam and China. *Viburnum punctatum* leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti periodic effect [6]. The different parts of the plant have been investigated phytochemically by several workers and found to contain sterols, terpenoids, sugars, glycosides and phenolic compounds. The plant has been reported to contain saponins, triterpenes in root, tannin, mucilage and lignin in leaf, saponins , starch grains and tannins in stem and terpenoid, glycoside and sterols in leaves [7] [8]. The research objectives were to investigate the antimicrobial activity of the chloroform and methanol extract from *V.punctatum* against standard and multi drug resistant gram positive and gram negative bacteria.

EXPERIMENTAL SECTION

Plant Material

Aerial parts of *Viburnum punctatum* were collected from Kalakkad-Mundenthurai, Thirunelveli in the month of June 2009. The plant was authenticated by Botanist. A voucher specimen of *Viburnum punctatum* (ABIPER/09/2013) was deposited in the department of Pharmacognosy in Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore for future reference. The plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and stored in an air tight and light resistant container for further use.

Preparation of Extracts

The coarsely powdered plant material was first defatted with Petroleum ether using soxhlet apparatus. The extract was concentrated using rotary evaporator to get solid residue. The marc from the central compartment was removed, dried and successively extracted with a series of solvents of increasing polarity with soxhlet extractor was done. Solvents used with increasing polarity are Chloroform, Methanol and Water.

Phytochemical Screening

The extracts of *Viburnum punctatum* were analysed for the presence of alkaloids, glycosides, flavonoids, fixed oils and fats, phenolic compounds and tannins, proteins and aminoacids, gums and mucilage, phytosterols and saponins according to standard procedures [9].

Alkaloids: 1.36gm of mercuric chloride dissolved in 60 ml and 0.5gm of potassium iodide in 10ml were dissolved in distilled water respectively. These two solvents were mixed and diluted to100ml using distilled water. To 1ml of acidic aqueous solution of sample add few drops of reagent. Formation of white or pale precipitate showed the presence of alkaloids.

Glycosides: A small amount of alcoholic extract of sample was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow color indicated the presence of glycosides.

Flavonoids: In a test tube containing 0.5ml of alcoholic extract the sample, 5-10 drops of diluted Hydrochloric acid and small amount of Zinc or Magnesium were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown color indicated the presence of flavonoids.

Fixed oils and fats: Few drops of 0.5M alcoholic potassium hydroxide was added to small quantities of extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Phenols: To 1 ml alcoholic solution of sample, 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color indicated the presence of phenols.

Tannins: In a test tube containing about 5 ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

Proteins and Amino acids: 1 ml sample was taken to that few drops Bradford reagent was added. The blue color was observed.

Phytosterols: Small quantities of various extracts were dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

1. Salkowski Test: To 1 ml above prepared chloroform solution, few drops of con.sulphuric acid was added. Brown color shows the presence of phytosterols.
2. Libermann Burchard Test: To above prepared chloroform solution, few drops of con.sulphuric acid followed by few drops of diluted acetic acid

Saponins: To 1 ml extract 5 ml of distilled water was added and shaken vigorously. Observed for soaking appearance indicates the presence of saponins.

ANTIBACTERIAL ACTIVITY

Well diffusion assay

The chloroform and methanol extracts were subjected to anti-bacterial activity by Agar diffusion assay is used widely to determine the anti-bacterial activity of crude extract. The technique works well with defined inhibitors. However when examining extract containing unknown components, there are problems leading to false positive and false negative results. Nutrient agar prepared and was poured in the Petri dish. 24 h growing *culture (Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli)* were swabbed on it. The wells (10mm diameter) were made by using cork borer. The different concentration (100 µg and 200 µg) of the crude extract were loaded in the wells. The plates were then incubated at 37ºc for 24 h. The inhibition diameter was measured [10].

ANTIFUNGAL ACTIVITY

Well diffusion assay

The chloroform and methanol extracts were subjected to anti-fungal activity. Potato dextrose agar prepared and was poured in the Petri dish. 24 h growing culture *(Candida albicans*) was swabbed on it. The wells (10mm diameter) were made by using cork borer. The different concentration (100 µg and 200 µg) of the crude extract were loaded in the wells. The plates were then incubated at 37ºc for 24 h. The inhibition diameter was measured [10].

Determination of Minimum Inhibitory Concentration (MIC)

In order to access biological significance and ability of the plant part, the minimum inhibitory activity was determined by Agar well diffusion method. Muller Hinton agar plate was prepared and overnight grown different species of bacteria such as *E.coli, S.aureus, S.typhi, K. Pneumoneae*, and *C.albicans* were swabbed. Wells approximately 10 mm was bored using cork borer and extracts of different concentration (10, 20, 30, 40, 60, 80µg) was added, the zone of inhibition was measured.

RESULTS AND DISCUSSION

The antimicrobial activity of chloroform and methanol extracts of *Viburnum punctatum* on different bacterial and fungal organisms have been shown Table 1.2 and 1.3. Antibacterial screening for the crude extracts were performed by agar diffusion assay against gram positive organisms *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus* and gram negative organism *Escherichia coli*. The zone of inhibition result was compared with standard drug Ciprofloxacin. Antifungal screening was carried out using the organism *Candida albicans* by agar diffusion assay method and activity was compared with the standard Ketoconazole.

1. Chloroform Extract of *Viburnum punctatum*- CEVP
2. Methanol Extract of *Viburnum punctatum*- MEVP

Table 1.1 Preliminary Phytochemical Screening of Pet ether, Chloroform, Methanol and Water Extracts

Test	Petroleum ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
Alkaloids	-	+	+	+
Glycosides	-	-	+	+
Flavonoid	-	+	+	+
Fixed oil and fats	+	-	-	-
Phenolic compounds, Tannin	-	+	-	-
Proteins and Amino acids	-	-	-	+
Gums and Mucilage	+	-	-	-
Phytosterol	+	+	-	-
Saponins	-	-	+	-

Table 1.2 Effect of CEVP and MEVP on Anti-Bacterial Activity

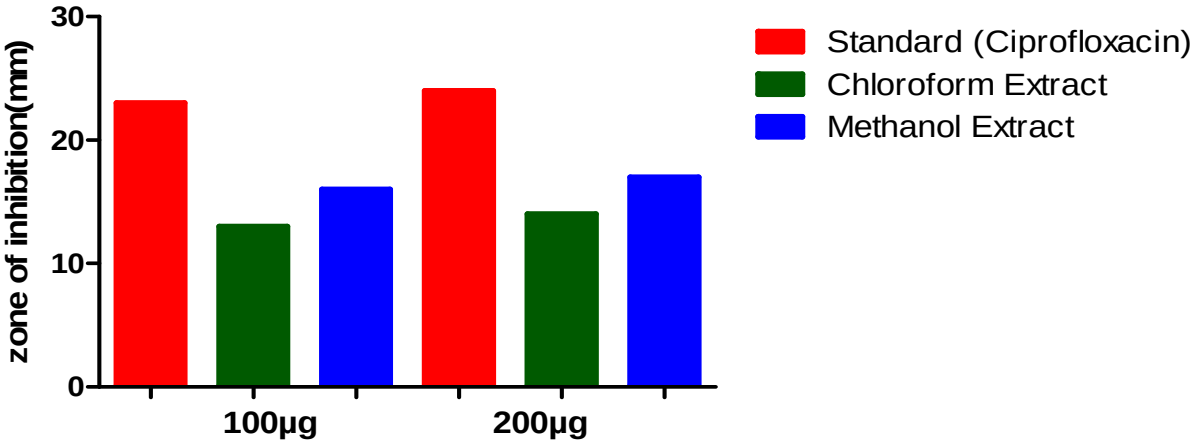
SI. NO	Name of the Extract	*S.typhi*		*S.aureus*		*K.pneumoniae*		*E.coli*	
		100µg	200µg	100µg	200µg	100µg	200µg	100µg	200µg
1.	Standard	23	24	24	26	23	25	24	26
2.	Chloroform Extract	13	14	12	13	13	15	12	14
3.	Methanol Extract	16	17	13	15	14	16	13	15

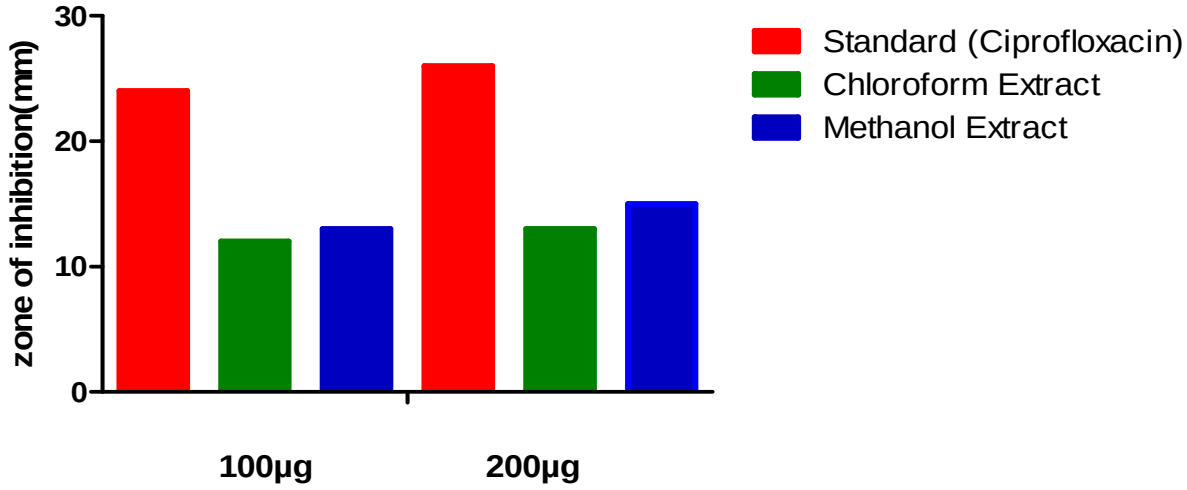
Table 1.3 Effect of CEVP and MEVP on Anti-fungal Activity

SI No	Name of the Extract	*C.albicans*	
		100µg	200µg
1	Standard	20	21
2	Chloroform Extract	12	15
3	Methanol Extract	13	16

Table 1.4 Determination of Minimum Inhibitory Concentration

Organism	MIC Value
Salmonella typhi	40µg
Staphylococcus aureus	80µg
Klebsiella pneumonia	40µg
Escherichia coli	40µg
Candida albicans	80µg





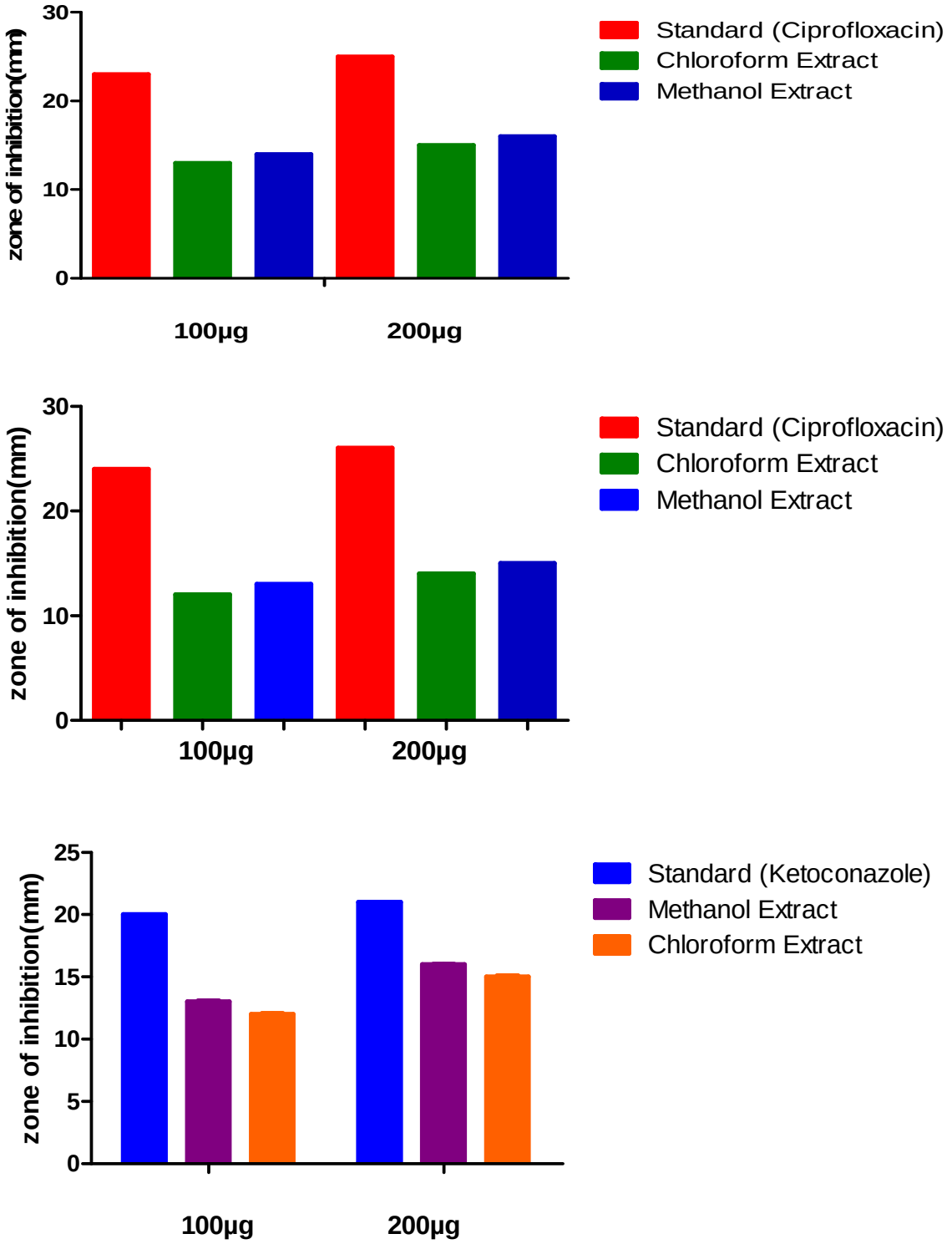


Figure 1.1 of CEVP and MEVP on Anti-Bacterial Activity- *S.typhi, S.aureus, K.Pneumoniae, E.coli* and *C.albicans*

In antimicrobial screening the methanolic extract exhibited good activity when compared with chloroform extract and zone of inhibition was closest value to the standard than the chloroform extract. The present study revealed that, *S.typhi* was highest susceptible bacteria with zone of inhibition 17mm followed by *K.pneumoniae* with zone of inhibition 16mm, *S.aureus* with zone of inhibition 15mm and *E.coli* with zone of inhibition 15mm. All results are depicted in Table 1.2. In antifungal screening, the methanolic extract also produced good antifungal activity with zone of inhibition 16mm than the chloroform extract. The observation results are depicted in Table 1.3.

Antibiotic usage for the prevention and treatment of bacterial infections in these high-risk patients leads to selection pressures resulting in the emergence and spread of resistant organisms. Many organisms acquire several resistances mechanisms; making them multi-drug-resistant [11]. Therefore the rapid propagation in antibiotic resistance and the increasing interest in natural products have placed medicinal plants back in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics [12].

Among the two extracts screened for antibacterial activity, the methanolic extract exhibited better antibacterial activity against *S.typhi, K.pneumoniae and E.coli* with an MIC value of 40µg and *S.aureus* with an a MIC value of 80µg. Then the methanolic extract exhibit good antifungal activity against *C.albicans* with an MIC value of 80µg. The results are depicted in Table 1.4.

CONCLUSION

The present investigation reported that the infections due to bacterial and fungal species. This study is report that the medicinal plant *Viburnum punctatum* used in Indian-herbal medicine may possess antimicrobial activity against *S.typhi, K.pneumoniae, E.coli, S.aureus, C.albicans* These findings can form the basis for further studies to toxicity testing, isolate active compounds, elucidate the structures, and also evaluate the antimicrobial study of the isolated compounds.

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