



## Comparative analysis on the *In-vitro* antibacterial activity of *hybanthus enneaspermus* F. muell. extracts against selected gram positive and gram negative bacterial strains

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### Abstract

The present study was investigated to *in vitro* antibacterial activity of aqueous, ethanol, methanol, petroleum ether and chloroform extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus salivarius*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Antibacterial activity of the pathogens was tested by disc diffusion assay method. Ethanol extract exhibited significant and broader spectrum of inhibition. *Proteus mirabilis* was highly inhibited pathogen among the selected pathogens. An attempt has been made to compare the activity of extracts with standard control (Streptomycin) against selected Gram positive and Gram negative pathogens.

**Keywords:** *hybanthus enneaspermus* F. muell, bacterial strains, *Staphylococcus aureus*

### Introduction

*Hybanthus enneaspermus* is a herbal plant used for medicinal purpose. It is also called as "hump back flower" and they are also called as green violet (L) F.muell. It is a genus of perennial often creeping whose leaves are alternate or in clusters (Priya *et al.* 2011) <sup>[1]</sup>. In Ayurveda, it is known as 'Sthalakamala'. The whole plant is used in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments (Krishnan *et al.*, 2012) <sup>[2, 7]</sup>. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretics, in urinary infection diarrhoea, leucorrhoea, dysuria and sterility (Yoganarasimhan, 2000). In some part of India, the plant is used to treat diabetes and is also having anti-oxidant property and free radical scavenging activity (Das, 2004) <sup>[4]</sup>.

The present investigation on various extracts of *Hybanthus enneaspermus* against selected Gram positive and Gram negative bacteria was a comparative study to expose the antibacterial activity of plant.

### Materials and Methods

The plant selected for the study *Hybanthus enneaspermus* (Linn.) F. Muell. was collected from Kanyakumari District, Tamilnadu. The plant was identified taxonomically. Fresh plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water. Then it was shade-dried, powdered and used for extraction. The extraction of solvents was as per the method followed by Akerele *et al.* (2008) <sup>[5]</sup> Human pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus salivarius*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Antibacterial activity of aqueous extract and solvent extracts (ethanol, methanol, petroleum ether and chloroform,) were determined by disc diffusion method on nutrient agar medium.

### Results and Discussion

Based on the zone of inhibition ethanol, methanol and chloroform extracts showed different degrees of antibacterial activity against gram positive and gram negative bacteria. Ethanol extract revealed the highest antibacterial activity against *Staphylococcus aureus*, *Streptococcus mutans* and *Proteus mirabilis*. Methanol extract was active against *Proteus mirabilis* and *Streptococcus mutans*. Similar findings of Napoleon *et al.* (2012) and Anand & Gokulakrishnan (2012) <sup>[2, 7]</sup> recorded ethanol and methanol as better solvent in their research works.

Chloroform extract showed antibacterial activity against *Klebsiella pneumonia*. Petroleum ether and aqueous extract was inactive against all the pathogens.

While comparing to the standard control, some of the pathogens were highly inhibited. Accordingly *Proteus mirabilis* was highly inhibited by ethanol extract and the zone of inhibition was 24mm, which shows higher activity than the standard control. Our results are in coincidence with the earlier findings of Murugan and Kamaraj (2018) <sup>[8]</sup> and Sahoo *et al.* (2006) <sup>[9]</sup>. Similarly few more organisms like *Staphylococcus aureus*, *Streptococcus mutans* and *Escherichia coli* also exhibited significant activity with higher zone of inhibition. Among the solvents least activity with 10 mm zone of inhibition was noticed in chloroform extracts.

**Table 1:** Antibacterial activity of selected Gram positive and Gram negative bacterial pathogens

		Ethanol	Methanol	Petroleum Ether	Aqueous	Chloroform	Positive Control
Gram +ve bacteria	<i>Staphylococcus aureus</i>	20	13	-	-	-	22
	<i>Bacillus subtilis</i>	15	12	-	-	10	22
	<i>Streptococcus mutans</i>	21	19	-	-	11	25
	<i>Enterococcus faecalis</i>	18	13	-	-	11	25
	<i>Lactobacillus salivarius</i>	18	16	-	-	10	21
Gram -ve bacteria	<i>Escherichia coli</i>	16	17	-	-	10	18
	<i>Klebsiella pneumonia</i>	13	12	-	-	14	20
	<i>Proteus vulgaris</i>	17	15	-	-	11	23
	<i>Proteus mirabilis</i>	24	20	-	-	13	22
	<i>Pseudomonas aeruginosa</i>	17	16	-	-	10	27

When different solvent extracts were compared ethanol extract of *Hybanthus enneaspermus* (Linn.) F. Muell. showed a marked antibacterial activity against *Proteus mirabilis* of gram negative strains followed by *Streptococcus mutans* and *Staphylococcus aureus* of gram positive strains.

The antibacterial activity exhibited by the plant extract was due to the presence of bioactive compounds such as phenols, flavonoids, glycosides, tannins, etc. Many researchers have worked on the phytochemical constituents of *Hybanthus enneaspermus* F. Muell. and found the compounds such as phenols, alkaloids, flavonoids, steroid, glycosides, tannins etc. (Anand and Gokulakrishnan, 2012, Anupa et al., 2016, Patel et al., 2011) [2, 7, 10, 11].

### Conclusion

The present study concluded that *Hybanthus enneaspermus* has potential antibacterial activity against most of the selected pathogens. *Proteus mirabilis* showed high inhibition than the standard control Streptomycin. Among the solvents ethanol extract inhibited most of the selected pathogens. Further studies must be needed in ethanol extract of *Hybanthus enneaspermus* against *Proteus mirabilis* for development of new pharmaceutical product.

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