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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT - *Hybunthus enneaspermus* L. F. Muell

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ABSTRACT

Hybanthus enneaspermus (L.). F. Muell is a small suffrutescent perennial herb distributed in the tropical and subtropical regions of the world. It grows 15-30 cm in height with many different diffuse or ascending branches and is pubescent in nature. The plant is popularly known as Rathanapurus or Pursharathna (Sanskrit, Hindi) and Orithazhthmarai(Tamil). This herb is considered to extremely beneficial to men, hence it is called Rathanapurush. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhea, leucorrhoea, dysuria and sterility. The plant kingdom shows many species of plant containing substances of medicinal value which have yet to be identified. Phytochemical analysis of major phytoconstituents which are possessing many biological activities, hence this study creates a presence of different extracts alkaloids, flavanoids, carbohydrates etc., The medical plants *Hybunthus enneaspermus* was from in an around Rasipuram, Namakkl district. Preliminary photochemical studies were performed for the presence or absence of Alkaloida, Proteins and Amino acids, Flavnoids, Phenolic compounds, Carbohydates and Phytosterol. Petroleum ether, Chloroform, Methanol, Ethanol and Isopropyl alcohol extracts of *Hybunthus enneaspermus* were investigated for antibacterial activity was tested against pathogenic bacteria. The petroleum ether extract inhibited maximum zones of inhibition were observed in *E. Coli*. Petroleum ether, Chloroform, Methanol and Ethanol extracts of *Hybunthus enneaspermus* were investigated for antifungal activity. The Methanol and Ethanol extract inhibited maximum zone of inhibition were observed in *Aspergillus niger and Aspergillus flavus.*

Keywords: Medicinal plant, Bacteria, Fungi, Phytochemical and *Hybunthus enneaspermus* L

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INTRODUCTION

Plants that are traditionally used in the treatment of bacterial and fungal infections or related ailments could offer potential lead in the development of novel herbal medicines that are active against pathogenic microorganisms. Many plants are active gaining importance due to fungi toxicity. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanism of action. They are effective in the treatment infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

In the recent past, there has been an increasing awareness of the beneficial values plants to man. Plant based drugs have got a wide application in folk medicine. *Hybunthus enneaspermus* (L). F.Muell is a rare ethano medicinal ephemeral herb[1] belongs to Violaceae. The plant is widely distribution in Africa, Madagascar, Srilanka, China, New Guinea, trophical Australia and India. The plant is moderately heightening herb of about 10 to 20 cms tall with a long slender tap

root. In view of its ethno medicinal importance, there is a need to conserve the wild stock of *Hybanthus enneaspermus*[2]. Plant extract contains and alkaloid, aurantiamide acetate, β-sitoserol and isoarborinol[3]. Vector containing high amount of amino acids including valine, leucine and gllutamic acid[4].

Plant extract contains and alkaloid, aurantiamide acetate, β-sitoserol and isoarborinol[3]. Vector containing high amount of amino acids including valine, leucine and gllutamic acid[4]. Assess the in vitro antimicrobial activity against *Bacillus subtilis, Escherichia coli, Staphylococcus auteus, Candida albians, Pseudomonas aeruginosa and Klebsiella pneumonia*. The ethanol extract exhibit significant and broader spectrum of inhibition in comparison to aqueous, moderate effect on the chloroform and petroleum ether extract. Compare the activity of extract with standard antibiotics against selected pathogen. Thus the plant was observed to have antimicrobial activity and Phytochemical compounds used for medicinal purposes[7].The Phytochemical analysis alkaloids, steroids and carbohydrates in leaf (*in vivo*) and the same metabolites also observed in leaf callus (*in vitro*)[8].

In the present investigation, efforts are made to find out the antifungal activity of different extracts of *Hybunthus enneaspermus* on the wet cloths with black and greenish black spots[9]. The aqueous extract of polyherb tested for antimicrobial efficacy against *Staphylococcus Sp, Micrococci Sp, Proteus Sp.,* and *Vibrio Cholerae., Proteus sp, Salmonella sp* found to be sensitive against pholyherb extract[10].

There was much variation in the results. Compared to aqueous extract lipohilic extracts showed good result with both the methods[11]. The antifungal activity of Hybanthus enneaspermus. Preliminary phytochemical screening revealed petroleum ether extract triterpenoids and steroids, chloroform extract steroids methanolic steroids, alkaloids, flavonoids, saponins, phenolic compound, amino acid, protein and tannins *Hybanthus enneaspermus* F. Muell. The anti-inflammatory, antitussive, antiplasmodial, anticonvulsant, free radical scavenging activity and antidiabetic[12].

Histopatological examination of rats hears section confirmed myocardial injury with isoproterenol. Administration of plant extract of *Hybunthus enneaspermus* reduced the oxidative stress by decreased lipid per oxidation and reduced glutathione normalized the levels of cardiac marker enzymes. *Hybunthus enneaspermus* –treated animals lesser degree of cellular infiltration in histopathological[13].

MATERIAL AND METHODS

Collection and identification of Medicinal plant

The selected medicinal plant *Hybunthus enneaspermus* (L). F. Muell. was collected from in an around Rasipuram, Namakkal district, Tamil Nadu, India and it was identified by Dr. E.G. Wesely, Botanist, AA Arts College, Namakkal.

Preliminary Photochemical Analysis

Various solvents extracts collected from the plants were tested for identification of its active chemical constituents.

Test for Alkaloids

To the small quantity of the test solution, a few drops of Dilute HCl was added and filtered. The filtered may be tested carefully with various alkaloidal reagents such as,

Mayor's reagent – Creamy Precipitate

Dragondroff's reagent – Orange Brown Precipitate

Hager's reagent – Yellow Precipitate

Wager's reagent – Reddish Brown Precipitate

Test for Proteins and Amino acids

Small quantities of test solution was dissolved in little quantity of water and treated with following reagents.

Millon's Reagent

Appearance of red color shows the presence of proteins and free amino acids.

Ninhydrin Reagent

Appearance of purple color shows the presence of proteins and free amino acids.

Biuret Test

Equal volume of 5% of sodium hydroxide and 1% solution of Copper Sulphate were added. Appearance of pink color shows the presence of proteins and free Amino acid

Picric acid test

Appearance of yellow color shows the presence of proteins and free amino acids.

Test for Anthraquinone glycoside

Borntrager's test

The small quantity of the test solution was boiled with diluted sulfuric acid and filtered. Ether was added to the filtrate and shaken well. The separated organic layer was added with ammonia. The layer became pink to red. It indicates the presence of Anthraquinone glycoside.

Test for Flavonoid

To the small quantity of the test solution, add aqueous sodium hydroxide solution, appearance of blue to violet color indicates the presence of anthocyanins, yellow color indicates the presence of flavones, yellow to orange indicates the presence of flavonoids.

Shimoda's test

The small quantity of the test solution is dissolved in alcohol, to the piece of magnesium followed by concentrated HCl drop was added and heated. Appearance of magenta colour shows the presence of Flavonoids.

Test for Tannin and Phenolic Compounds

The small quantity of the test solution was taken separately in water and tested for the presence of Phenolic compounds and tannins with following reagents such as

Diluted Ferric Chloride solution (5%) – Violet Color

1% Solution Gelatin containing 10% NaCl – White Precipitate

10% Lead Acetate solution – White Precipitate

Test for Carbohydrates

The small quantity of test solution was dissolved in 5ml of distilled water and filtered. The filtrate was subjected for carbohydrates.

Molisch' test

The filtrate was treated with 2 – 3 drops of 1% alcoholic alpha napthol, and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of purple colour ring indicates the presence of carbohydrates.

Fehling's test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Anthrone test

The filtrate was treated with anthrone reagent and dil. Sulphuric acid. Red color shows the presence of carbohydrates.

Test for Saponins

The small quantity of test solution was diluted with 20 ml of distilled water and it is agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by the formation of 1cm layer of foam.

Test for Phytosterol

Liebermann Burchard test

The small quantity of test solution was dissolved in a few drops of dry acetic acid; 3 ml of acetic anhydrate was added followed by few drops of concentrated sulfuric acid. Appearance of bluish green color indicates the presence of Phytosterols.

Test for terpenes

To the small quantity of test solution add thionyl chloride appearance of pink colour shows the presence of Terpenes.

Test for Coumarin

To the small quantity of test solution add 10% NaOH solution. Appearance of yellow colour shows the presence of coumarin.

Test for Quinone

To the small quantity of test solution add Conc H_2SO_4.Apperance of red color shows the presence of Quinone.

Test for Starch

To the small quantity of test solution add iodine solution. Appearance of blue color indicates the presence of Starch.

Test for Gum

To the small quantity of extract add drop of water, if it swells or showing adhesive nature indicates the presence of Gum.

Collection of test organism

The bacterial culture namely *Escherichia coli* MTCC-443, *Bacillus subtilis* MTCC-441, *Staphylococcus aureus* MTCC-796, *Proteus mirabilis* MTCC-442, *Klebsiella pneumoniae* MTCC-109, and the bacterial culture were obtained from Agriculture University Coimbatore, Tamilnadu.

Antimicrobial efficacy of *Hybunthus enneaspermus* (Linn.)

Hybunthus enneaspermus was powdered and extracted in soxhlet apparatus successively with petroleum ether, methanol, ethanol, chloroform, isopropyl alcohol. The extracts were tested for antimicrobial activity against Gram negative and gram positive bacteria.

Nutrient agar Medium

The bacterial culture was inoculated into Nutrient agar medium and incubated for 18-24 hours at 37ºC, and observed the results.

Media Composition/Litre

Peptone - 5.0g, NaCl -5.0g, Yeast Extract - 2.0g, Agar - 15g, pH - 7.4±0.2 at 25ºC .

Muller Hinton agar medium

Inoculums of the five bacterial strains was inoculated with muller hinton agar medium and incubated for 18-24 hours at 37ºC after the incubation the result could be observed

Beef, infusion from casein acid -300.00g,Hydrolysate - 17.50g, Starch-1.50g, Agar-17.50g,pH-7.3±0.2 at 25ºC

Antibacterial activity

The antibacterial activity of the above mentioned extracts were determined, using method. The bacteria test organisms were growing in nutrient broth for 24 hours. A 100ml of the nutrient broth culture of each bacterial organism was used to prepare bacterial lawn. Agar were prepared and loaded with 100µl of plant extract. Each plate was used as a control and loaded with 100µl of solvent. The plates were incubated at 37ºC. An attempt has been made to compare antibacterial activity of *Hybanthus enneaspermus* extract with the most potent standard antibiotics against selective pathogen.

Collection of test organism

The fungal culture namely *Aspergillus niger* MTCC - 281, *Aspergillus flavus* MTCC-277, *Penicillin notatum* MTCC-2011, *Rhizoctoni solani* MTCC-4633, *Colletrotrichum falcatum* 10291, and the fungal culture was obtained from Agricultureunvirsity Coimbatore tamilnadu,

Antifungal activity

The antigungal activity of the above mentioned extracts were determined the method. The fungi test organisms were growing in Potato dextrose agar medium for 48 hours. A 100 ml of the potato dextrose culture of each fungi organism was used to prepare fungi lawn. Agar were prepared and loaded with 50, 100µl of plant extract. Each plate was used a s a control and loaded 100µl of solvent. The plates were kept in overnight culture. An attempt has been made to compare antifungal activity of *Hybanthus enneaspermus* extract with the most potent standard antibiotics against selective pathogen.

Potato Dextrose agar medium- Potato (peeled)-0.200g,Dextrose-20.0g, Agar -15.0g, Distilled water-1 Lit,pH -6.2.Peeled off potatos were cut into small bits and boiled in 50 ml of water, filtered through cheese cloth and dextrose was added to filtrate . Agar was dissolved in water added to the mixture and made up to one litre.

Potato dextrose agar medium was used in this study. *Aspergillus niger* was grown MHA broth for 3 days. Fungal culture was spread on the agar surface using sterile cotton swab. The wells in each plate were loaded with 100µl of plant extract. The centre well in each plate was used as a control and loaded with 100µl of solvent. The plates were incubate at 27ºC for 2 days

RESULT

Preliminary Phytochemical analysis were performed for the presence or absence of Alkaloids, Proteins and Amino acids, Flavnoids, Anthraquione glycosides, Tannins, Phenolic Compounds, Saponins, Carboydrates, Phytosterols, Terpenes, Coumarin, Quinone, Gum and Starch in different plant extracts plant extracts were studies.Chloroform extract of leaves showed the presence of Alkaloids, Flavoniods, Carbohydrates and Phytosterol. Absence of Protein and Amino acids. Methanol extract of leaves showed the presence of Alkaloids, Carbohydrates, and Phytosterol. Absence of Phenolic compound. Ethanol extract of leaves showed the presence of Alkaloids, Flavanoids and Carbohydrates. Absence of Phytosterol. Petroleum ether extract of leaves showed the presence of Alkaloids, Flavnoids and Carbohydrates. Absence of Phytosterol, Proteins and Phenolic compounds. These preliminary phytochemical screening results of this plant result were tabulated (Table 1).

Table 1: Qualitative Phytochemical screening of various solvent extracts of leaves of *Hybunthus enneaspermus.*

S. No	Tests	Chloroform	Methanol	Ethanol	Petroleum eather
1.	**Alkaloids**				
	Mayer's test	+	+	+	+
	Drgendorff's test	+	+	+	+
	Hangers test	+	+	+	+
	Wagers test	+	+	+	+
2.	**Proteins and amino acids**				
	Millions test	-	+	-	-
	Ninhydrin test	-	+	-	-
	Biruet test	-	+	-	-
	Picric acid test	-	+	-	-
3.	**Flavonoids**				
	Shimoda's test	+	+	+	+
4.	**Phenolic Compounds**				
	Ferric chloride test	-	-	-	-
	Tenning test	-	-	-	-
5.	**Carbohydrates**				
	Molischs test	+	+	+	+
	Feling's test	+	+	+	+
	Anthrone test	-	+	-	-
6.	**Phytosterol**				
	Liebermann Burchard	-	-	-	-
	Terpenes	-	-	-	-
	Coumarin	+	+	-	+
	Strach	-	-	-	-
	Gum	-	-	-	-

Petroleum ether, Chloroform, Methanol, Ethanol and Isoproyl alcohol extracts of *Hybunthus enneaspermus* were investigated for their antibacterial was tested against pathogenic bacteria at different concentrations of 25, 50, 75, 100µl/ml results were tabulated. The *Hybunthus enneaspermus* zone of inhibition observed (Table 2). It appeared that the extracts inhibited the zone of bacterial growth of all the test bacteria at different concentrations (25, 50, 75,100µl/mg) to varying degree. The Extracts exhibited prominent inhibitions of zone against all the test fungi except with 50 and 100µl/ml. The growth of *B. subtilis, E. coli, S. aureus, Proteus mirabilis* and *Klebsiella pnumoniae* was inhibited considerably particularly at higher dose (100µg/ml disc). The growth of bacterial zone in inhibition using petroleum ether extracts against *E. coli, B. subtilis, S. aureus, Proteus mirabilis* and *Klebsiella pnumoniae* were 13mm,15mm,18mm,19mm and 18mm respectively at 100µg/ml (Table 2-7). The petroleum ether extract inhibited maximum zones of inhibition were observed in *E. coli*.

Table 2: Antibacterial activity of chloroform extracts of *Hybunthus enneaspermus* against pathogenic bacteria

S.No	Microoraganisms	Zone of inhibition (in mm)			
		25µl	50µl	75µl	100µl
1.	*Escherichia coli*		11	12	13
2.	*Bacillus subtilis*	11	12	13	14
3.	*Staphylococcus aureus*	12	13	14	19
4.	*Proteus mirabilis*	11	12	13	14
5.	*Klebsiella pneumoniae*	12	13	15	16

Table 3: Antibacterial activity of Methanol extracts of *Hybunthus enneaspermus* against pathogenic bacteria.

S.No	Microoraganisms	Zone of inhibition (in m			
		25µl	50µl	75µl	100µl
1.	*Escherichia coli*	11	12	13	15
2.	*Bacillus subtilis*	12	13	14	16
3.	*Staphylococcus aureus*	12	13	15	16
4.	*Proteus mirabilis*	12	12	13	14
5.	*Klebsiella pneumoniae*	12	13	14	15

Table 4: Antibacterial activity of Ethanol extracts of *Hybunthus enneaspermus* against pathogenic bacteria

S.No	Microoraganisms	Zone of inhibition (in mm)			
		25µl	50µl	75µl	100µl
1.	*Escherichia coli*	12	13	14	17
2.	*Bacillus subtilis*	12	13	14	15
3.	*Staphylococcus aureus*	12	13	14	17
4.	*Proteus mirabilis*	11	13	14	16
5.	*Klebsiella pneumoniae*	12	14	15	20

Ethanol, Methanol, Chloroform, Petrolium ether and Isoprpyl alcohol extracts *Hybunthus enneaspermus* were investigated for their antifungal activity was treated against pathogenic fungi at different concentraticons of 50,100 mg/ml and the results were tabulated. The inihibition of fungal growth of petroleum ether extracts of *Hybunthus enneaspermus*.It appeared that the extracts inhibited against all the test fungi at different concentrations (50 to 100mg/ml). The highest inhibition of fungal radial growth was recorded against all the test fungi at concentration of 100mg/ml medium using the petroleum ether extracts

Table 5: Antibacterial activity of Petroleum ether extracts of *Hybunthus enneaspermus* against pathogenic bacteria.

S. No	Microoraganisms	Zone of inhibition (in mm)			
		25µl	50µl	75µl	100µl
1.	*Escherichia coli*	11	12	12	13
2.	*Bacillus subtilis*	12	14	17	19
3.	*Staphylococcus aureus*	11	13	14	18
4.	*Proteus mirabilis*	11	12	13	15
5.	*Klebsiella pneumoniae*	12	12	16	18

Table 6: Antifungal activity of Chloroform extracts of *Hybunthus enneaspermus* against pathogenic fungi.

S.No	Microoraganisms	Zone of inhibition (in mm)	
		50µl	100µl
1.	*Aspergillus niger*	20	26
2.	*Aspergillus flavus*	19	28
3.	*Penicillin notatum*	22	36
4.	*Rhizoctonia solani*	12	24
5.	*Colletrotrichum falcatum*	26	38

Table 7: Antifungal activity of Methanol extracts of *Hybunthus enneaspermus* against pathogenic fungi.

S.No	Microoraganisms	Zone of inhibition (in mm)	
		50µl	100µl
1.	*Aspergillus niger*	12	21
2.	*Aspergillus flavus*	18	25
3.	*Penicillin notatum*	13	26
4.	*Rhizoctonia solani*	12	20
5.	*Colletotrichum falcatum*	24	31

Table 8: Antifungal activity of Ethanol extracts of *Hybunthus enneaspermus* against pathogenic fungi.

S.No	Microoraganisms	Zone of inhibition (in mm)	
		50µl	100µl
1.	*Aspergillus niger*	20	25
2.	*Aspergillus flavus*	25	30
3.	*Penicillin notatum*	25	32
4.	*Rhizoctonia solani*	20	25
5.	*Colletotrichum falcatum*	36	40

Table 9: Antifungal activity of Petroleum ether extracts of *Hybunthus enneaspermus* against pathogenic fungi.

S.No	Microoraganisms	Zone of inhibition (in mm)	
		50µl	100µl
1.	*Aspergillus niger*	13	20
2.	*Aspergillus flavus*	15	22
3.	*Penicillin notatum*	14	21
4.	*Rhizoctonia solani*	10	16
5.	*Colletotrichum falcatum*	21	28

The present *Hybunthus enneaspermus* growth inhibition of the fungi at different concentrations of methanolic extract of leaves of *Aspergillus niger, Aspergillus flavus, Penicilin notatum , Rhizoctonia solani, Colletotrichum falcatum,* was observed at 100mg/ml concentration of the methanolic extract, compared with control. The percent inhibition of *Aspergillus niger* is 21% and *Aspergillus flavus* 25%. It is observed that the inhibition was concentration dependent and that higher inhibition of growth was observed at 100 mg/ml concentration. Percent inhibition of radial growth of ethanolic extract against *A.niger, A.flavus, Penicillin Notatum, Rhizoctonia solani, Colletrotrichum falcatum* (Table.7-9)

DISCUSSION

In previous studies the Screening of plant extract for phytochemical analysis for the presence of alkaloid, flavonoid, tannins, saponins, etc., *Hybunthus enneaspermus* leaves possess antimicrobial properties mainly due to the presences of Phytochemical compound[14,15,16,17]. Thus it can be used for medicinal purpose. In this present study standardized the presence of flavonoids, Alkaloids, Protins, amino acids, Carbohydrates, phenols and tannins. In this Phytochemical responsible for the observed antimicrobial property[18,19,20,21].

The ethanol and methanol leaf extract of *Hybunthus enneaspermus* showed potent antimicrobial activities against the test pathogens. Ethanol extract showed more significant activity with maximum zone of inhibition 20mm, against *S.aureus* and minimum (10 mm) against *B. sutilis* and *Proteus*. Chloroform extract showed inhibition against *S. aureus, Proteus mirabilis, and K. pnumoniae respectively* 19mm, 14mm, 16mm. Petroleum ether extract was active against *K. pnumoniae* 18 mm. similar studies was carried out by several author.[22,23,24,25]

In the present study Ethanol, Methanol, Chloroform, Petrolium ether and Isoprpyl alcohol extracts of *Hybunthus enneaspermus* showed potent[26,27,28].

Antifungal activity against the pathogens Methanol extract possess more Zone of inhibition *Colletrotrichum falcatum* (31mm), *Aspergillus niger* (21mm), and minimum zone of *Aspergillus niger* (12mm), Ethanol extract also showed significant activates against *Colletrotrichum falcatum* (40mm) and minimum is *Aspergillus niger*, *Rhizoctonia solani* (20mm). Similar study was carried out by author. [26,27,28,29] From this present investigation *Hybunthus enneaspermus* (L). F. Muell is one of the best medicinal plants having growth antimicrobial activity [30,31,32,33,34].

CONCLUSION

Preliminary photochemical studies were performed for the presence or absence of Alkaloida, Proteins and Amino acids, Flavnoids, Phenolic compounds, Carbohydates and Phytosterol. Petroleum ether, Chloroform, Methanol, Ethanol and Isopropyl alcohol extracts of *Hybunthus enneaspermus* were investigated for antibacterial activity was tested against pathogenic bacteria. The petroleum ether extract inhibited maximum zones of inhibition were observed in *E. Coli*. Petroleum ether, Chloroform, Methanol, Ethanol and Isopropyl alcohol extracts of *Hybunthus enneaspermus* were investigated for antifungal activity. The Methanol and Ethanol extract inhibited maximum zone of inhibition were observed in *Aspergillus niger and Aspergillus flavus.*

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