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Phytochemical screening and antimicrobial resistance of *Alseodaphne semecarpifolia* nees

Charles A*and Alex Ramani V

PG & Research Department of Chemistry, St.Joseph's College, Trichy, Tamil Nadu, India

ABSTRACT

Ethanolic leaves extract of Alseodaphne semecarpifolia subjected to successive extraction using the solvents like n-hexane, toluene, chloroform, ethyl acetate and methanol. Prepared various solvents leaves extracts were then subjected to preliminary phytochemical and biochemical studies. It was found that the leaves extract contains steroids, alkaloids, phenolics, flavonoids, glycosides, essential oils, tannins and saponins etc. The ethanolic extract was selected for the antimicrobial activity against different pathogens of 20 bacteria and 6 fungus and it was determined by using Agar Well diffusion method. Moreover, this extract showed inhibitory effects due to the above mentioned phytochemicals present in the leaves.

Key words: Alseodaphne semecarpifolia, Phytochemical screening, Antimicrobial, Agar Well diffusion method.

INTRODUCTION

Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound [1].

Alseodaphne semecarpifolia belongs from Lauraceae (Laurel family) it is commonly known as Nelthare; in Tamil kanaippirandai. Nelthare is a large evergreen tree up to 18 m tall, found in peninsular India. Bark is brownish, scaly and flaky. Leaves are alternately, spirally arranged and clustered at twig ends. Leaf stalks are stout 0.7-2 cm long. Leaves are 7-16 cm long, 4-8.5 cm broad, obovate, tip blunt or rounded, sometimes notched. Leaf base is wedge-shaped. Leaves are leathery, hairless, glaucous beneath. Midrib is slightly raised above, parasitic vine with leaves reduced to scales, usually aromatic. Tiny yellowish flowers are borne in panicles at the end of branches, 10-20 cm long. Flowers have 6 petals which fall off. The fruit is black, round, 1-2 cm across. [2-4] [Fig.1]

A. semecarpifolia in ethno veterinary practices in India the stem bark is used for Rinderpest disease, dysentery in cattles[5] and also juice is applied externally for leach bite [6]. In the present study, we have concentrated on the phytochemical screening by using of various solvents leaves extract of A. semecarpifolia by fractionating followed by concentrated ethanolic extract. In order to find out the active biochemicals and phytochemicals like steroids, alkaloids, triterpenoids, Proteins, amino acids, phenolics, flavonoids, glycosides, essential oils, tannins and saponins etc.,

The ethanolic leaves extract of A. semecarpifolia were selected for the antimicrobial activity against different pathogens of 20 bacteria and 6 fungus was determined by Agar Well diffusion method or cork borer method. Antiseptics, disinfectants and antibiotics are used in different ways to combat microbial growth. Antiseptics are used on living tissue to remove pathogens. Disinfectants are similar in use but are used on inanimate objects. Especially, this method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body.

EXPERIMENTAL SECTION

Collection and identification of plant material.

The Leaves of A. semecarpifolia were collected from the Kolli Hills, in Namakal Disrtict, Tamilnadu, India, in Jan 2011.The plant was identified and it was authenticated with vouch specimen by Rapinant Herbarium, St.Joseph'College, Trichy, Tamilnadu, India.

Phytochemical screening procedure.

Preparation of extract.

The air dried leaves of A. semecarpifolia (1kg) were extracted with 80% ethanol (4x500) under reflux. The alcoholic extract was concentrated in vacou for further fractionated successively with n-hexane, (2x300), toluene (2x100), chloroform (2x300) ethyl acetate (2x500) and methanol (2x100) solvents were then removed under reduced pressure. Phytochemical analysis of different solvent extracts subjected to following methodology of Harborne [7] shown in Table 1 for preliminary phytochemical screening.

Preliminary screening for anti bacterial and anti-fungal activity

Test organisims
The following organisims were employed for this study as test organisims:

Bacteria
Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Shigella sp, Clostridium septicum, Clostridium tetani, Bifidobacterium bifidum, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella enteritidis, Klebsiella pneumonia, Enterobacter sp., Proteus mirabilis, Pseudomonas mutant, streptococcus sp., Proteus vulgaris, Bacillus substilis mutant, Yersinia.[8-11]

Fungi
Aspergillus niger, A. flavus, A. nidulans, A. oryzae, Penicillium sp, Mucor. The bacterial and fungal pathogenic strains were obtained from the Amphigene research laboratories, Thanjavur, Tamilnadu, South India.

preparation of inoculum
Using sterile inoculation loop 20 pure colonies of the test organism are transferred to 5ml of sterile nutrient broth and incubated at 37 ^{0}C overnight for 18hrs. Then this bacterial culture were suspended in saline solution (0.85%Nacl) and adjusted to a turbidity of 0.5 Mac Farland standards (10^8cfu/ml). This suspension was used for preliminary screening of anti bacterial activity.

Agar well diffusion assay
The modified agar well diffusion method of Perez et al. [12], was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts and blanks (ethanol, distilled water, and n-hexane). The concentration of the leaves extracts of A. semecarpifolia employed was 25 µg/ml. The test was carried out by triplicate. The plaques were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated by applying the expression in mm as shown in Table 2. The graphical representation of the Zone of Inhibition as shown in Fig. 2, 3, and Fig 4.

RESULTS AND DISSUSION

The results obtained in the present investigation [Table 1], showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, triterpenoids and the absence of proteins and amino acids. Different active phytochemicals have been found to possess a wide range of activities, which may help in the protection against incurable diseases. This study is only a preliminary study of the occurrence of certain properties of A. semecarpifolia leaves an in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above.

The concentrated ethanolic extract leaves of A. semecarpifolia was subjected to further evolution of antimicrobial activities against different pathogens of 20 bacteria and 6 fungus was

determined by Agar Well diffusion method. The Zone of Inhibition of Bacteroides melaninogenicus, Staphylococcus aureus and Streptococcus sp. (19mm) is maximum with in the 20 bacteria whereas Bacteroides fragilis and Klebsiella pneumoniae is minimum inhibition are recorded and tabulated at a concentration of 25µg/25µl in Table 3.Among the 6 Fungus, Aspergillus niger only showed the activity (14mm) at a concentration of 25µg/25µl.

CONCLUSION

In this present study, we have found that biologically active biochemicals and phytochemicals which were present in the solvents like n-hexane, toluene, chloroform, ethyl acetate, chloroform and methanol extract of A. semecarpifolia leaves. The antimicrobial potentials of ethanolic leaves extracts may be due to the presence of the above mentioned active biochemicals and phytochemicals. Further studies are in progress in our laboratory to isolate the active components from the leaves of A. semecarpifolia.

Fig. 1 Alseodaphne semecarpifolia

Figure: 2. Zone of Inhibition of A. semecarpifolia Leaves extracts against selected Microorganisms

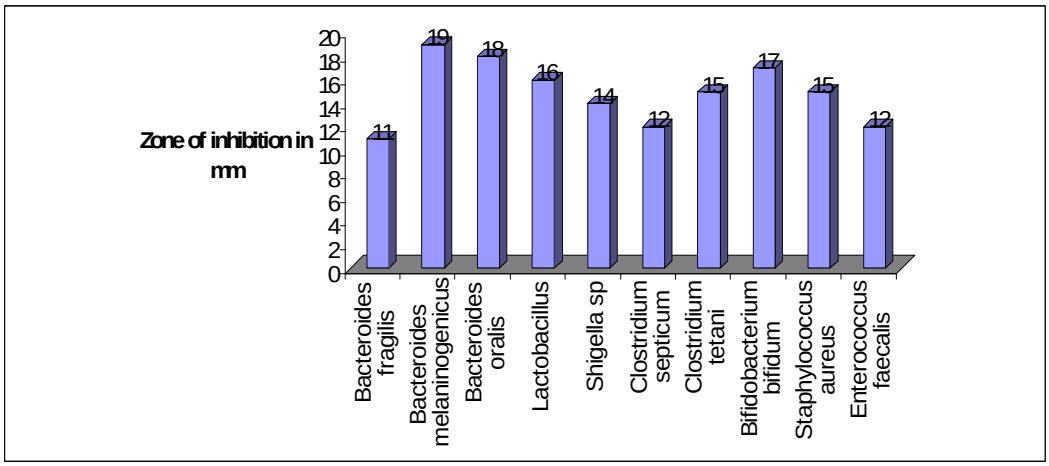

Figure: 3. Zone of Inhibition of A. semecarpifolia Leaves extracts against selected Microorganisms.

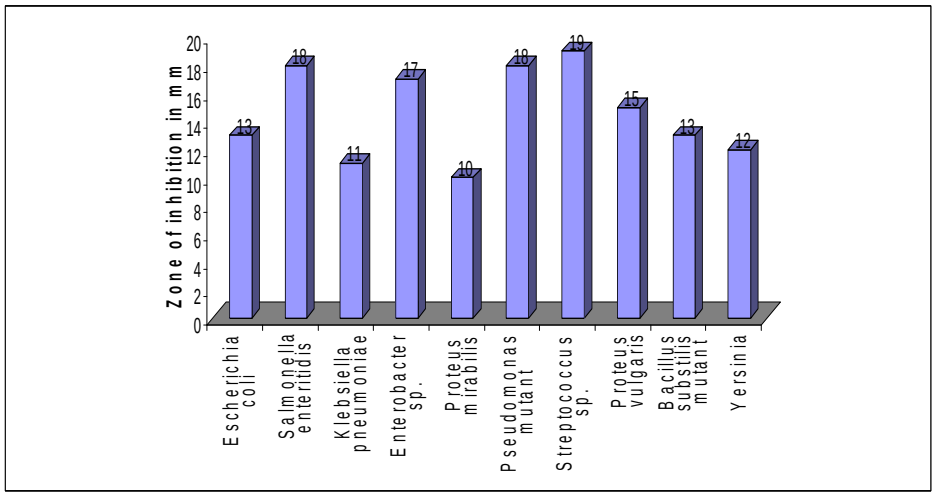

Table 1. Phytochemical analysis of different leaves extracts of A. semecarpifolia

S. NO	NAME OF THE TEST	PROCEDURE	OBSERVATION	H	T	C	EA	M
1	Alkaloids	Extact + Dragondroffs reagent Mayer's reagent Hager's reagent	 Orange color White ppt Yellow ppt	 - - -	 - - -	 - - -	 - - -	 + + +
2	Glycosides	Anthrone + H2SO4+Heat	Purple or green	-	-	-	+	+
3	Carbohydrates	Extact + Molish's reagent+ conc.H2SO4 Fehling's solution A&B	Purple color Brick red color	- -	- -	- -	+ +	+ +
4	Phytosterols /triterpenoids	LiebermannTest Salkowski Test Noller's test	Bluish green Red fluorescent Pink color	+ + +	+ + +	- - -	- - -	- - -
5	Proteins & Amino acids	Biuret test Xanthoprotein test Millon's reagent test Ninhydrin test	Violet color Orange color White ppt White ppt	- - - -	- - - -	- - - -	- - - -	- - - -
6	Saponins	Extact + water+ shaking	Formation of honey comb like froth	-	-	-	-	+
7	Flavonoids	Shinoda's test Zn-HCI acid reduction test	Red colour Magenta color	- -	- -	+ -	+ +	+ +
8	Fixed oils & Fats	Spot test	Stains appear after drying	+	+	-	-	-
9	Gums/Mucilage	Extact +water	No thickening of the substance	-	-	-	-	+
10	Volatile oil	-	-	+	-	-	-	-
11	Phenolics/ Tannins	FeCl3 Extract + lead acetate+ water	Intense color Formation of white ppt	- -	- -	+ +	+ +	- +

H = n-Hexane, T = Toluene, C = Chloroform, EA = Ethyl acetate, M = Methanol.

- = absent + = present

Fig 4. Evaluation of Antifungal activity of ethanolic Leaves extract of A. semecarpifolia

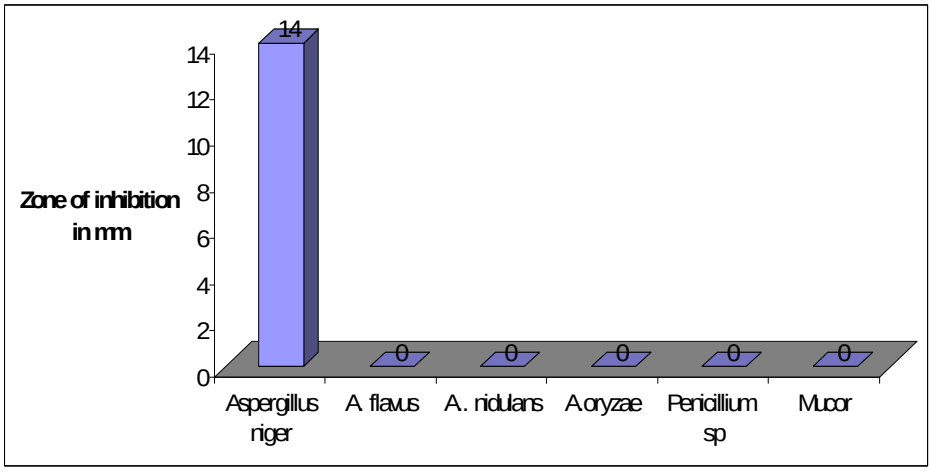

Table: 2. Zone of Inhibition of A. semecarpifolia Leaves extracts against selected Microorganisms

S.No	Name of Pathogens	Control	Zone of inhibition (mm)	S. No	Name of Pathogens	Con-trol	Zone of inhibition (mm)
1	*Bacteroides fragilis*	0	11	11	*Escherichia coli*	0	13
2	*Bacteroides melaninogenicus*	0	19	12	*Salmonella enteritidis*	0	18
3	*Bacteroides oralis*	0	18	13	*Klebsiella pneumoniae*	0	11
4	*Lactobacillus*	0	16	14	*Enterobacter sp.*	0	17
5	*Shigella sp*	0	14	15	*Proteus mirabilis*	0	10
6	*Clostridium septicum*	0	12	16	*Pseudomonas mutant*	0	18
7	*Clostridium tetani*	0	15	17	*Streptococcus sp.*	0	19
8	*Bifidobacterium bifidum*	0	17	18	*Proteus vulgaris*	0	15
9	*Staphylococcus aureus*	0	15	19	*Bacillus substilis mutant*	0	13
10	*Enterococcus faecalis*	0	12	20	*Yersinia*	0	12

Table: 3. Evaluation of Antifungal activity of ethanolic Leaves extract of A. semecarpifolia

S. No.	Name of Species	Control	Zone of inhibition (mm)
1	*Aspergillus niger*	0	14
2	*A. flavus*	0	Nill
3	*A.. nidulans*	0	Nill
4	*A.oryzae*	0	Nill
5	*Penicillium sp*	0	Nill
6	*Mucor*	0	Nill

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