Preliminary phytochemical and Antimicrobial Studies on the Leaf Extracts of *Actinodaphne lanata* Meissner.

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ABSTRACT:

The present study was carried out to evaluate the antimicrobial potential of *Actinodaphne lanata* Meissner (Lauraceae) from leaf extract. Solvent petroleum ether, ethyl acetate, Methanol and aqueous extracts were tested against the test organisms viz., Bacterial stains (*Streptococcus pygenes, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, E.coli* and *Pseudomonas aeruginosa)* and fungal stains (*Aspergillus niger, A.flavus, Fusarium oxysporum and Candida albicans*).Methanol extract of *A.lanata* had maximum zone inhibition against *Streptococcus pygenes,* Where as ethyl acetate extract of *A.lanata* showed maximum zone inhibition against *Klebsiella pneumonia*. Aqueous extract of the plant at different concentration showed less inhibition on the tested organisms. Phytochemical analysis recorded positive results for alkaloids, phenols, tannins, saponins and terpenoids. Among the various extracts methanol extract of the investigated plant leaves of *Actinodaphne lanata* was found to more effective against all the pathogens. The results of these studies revealed most valuable information and also support the continued sustainable use of this plant in traditional systems of medicine.

Keywords: Phytochemical screening, anti-bacterial activity, *Actinodaphne lanata*

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) reports that about 80% of the World's population depends on traditional medicines to meet at least some of their primary healthcare[1]. Plants produce an incredible array of secondary metabolites and many of these have been developed into economically important products including; oils, gums, resins, tannins, rubber, waxes, pigments, flavors, fragrances, surfactants, preservatives, pesticides and pharmaceuticals[2].Thus, medicinal plants are under tremendous pressure all across the globe, especially in India. More than 90% of the medicinal plants for herbal industries in India and for export are drawn from the natural habitats thus challenging their existence [3,4].The structure of flavonoid compounds is a key determinant of their radical scavenging and metal chelating activity, and this is referred to as structure-activity relationships[5].Historically plants have provided a good source of anti-infective agent[6-7]. Medicinal plants are finding their way into pharmaceuticals, neutralceuticals, cosmetics and food supplements. Infections diseases account for approximately one –half-of all deaths in tropical counties. Incidence of epidemics due to drug resistant micro-organisms and emergence of hitherto unknown pathogenic microbes pose enormous public health concerns[8].

The genus Actinodaphne belongs to the family Lauraceae with about 100 species occurs mainly in tropical-subtropical Asia and is an important component of tropical forests. *Actinodaphne lanata* Meissner of family Lauraceae is tall tree, evergreen (or) rarely deciduous between 1500 and 1800 m. Endemic to Nilgiris Biosphere Reserve and critically endangered (Watler and Gillett, 1998. Actinodaphne genus is one of the plants which have been used in traditional medicine for many years. Therefore, this study is designed to test for the activities of the ethyl acetate and methanol leaf extracts of *Actinodaphne lanata* against three species of Gram –ve and three species of Gram +ve bacteria strains and fungal. The results of the preliminary phytochemical analysis will provide suggestions as to the probable secondary metabolites responsible for the activities of the extracts.

Material and Methods:

Plants Material

The leaves of *Actinodaphne lanata* were collected from Tropical Gene pool Garden, of Gudalur, Western Ghats of Nilgirs district, Southern India. The samples of plant were identified Tropical Gene pool Garden and binomially by Botanical survey of India (Southern part coimbatore, Tamil Nadu, India) and voucher specimens were deposited at the Herbarium Departments of Tamil Nadu forest department, Gene pool Garden, Nadugani, Gudalur, Nilgirs , Tamil Nadu, India.

Extraction of plant Material

Healthy fresh leaves of *Actinodaphne lanata* was collected from tropical Gene Pool Garden, Nilgiris district. The leaves were shaded dried and powdered . 50g of fine powder was packed with what man No.1 filter paper and placed is soxhlet apparatus along with solvent petroleum ether and followed by methanol. The residues were collected and dried at room temperature 30^0C after which yield was weighed and then performed to activity .

Primary phytochemical investigation

The preliminary bioactive phytochemical constituents were tested by following methods. The extracts were screened for the presence of phenol[11], flavonoids[12], alkaloids[13,14,15], tannins[16],saponins, steroids, terpenoids, glycosides, cardiac glycosides and reducing sugar[17-18].

Antibacterial activity

Bacterial pathogens

Antimicrobial activity of crude extract was tested against bacterial pathogens belong three gram-positive bacteria such as *Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilis* and three gram negative bacteria such as *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were obtained from PSG medical college, Coimbatore, Tamilnadu, India. They were grown in nutrient broth medium and incubated at 37ºC for 48 h followed by frequent sub culturing (at every 48 h) on to the refresh medium.

Fungal pathogens

Fungal pathogens such as *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum* and *Candida albicans* were obtained from PSG medical college, Coimbatore, Tamilnadu, India. They were grown in nutrient broth medium and incubated at 37ºC for 48 h followed by frequent sub culturing (at every 48 h) on to the refresh medium.

Antimicrobial activity (Disc diffusion method)

The agar diffusion method was used to evaluate the antimicrobial effect of the leaf extracts**.** Inoculums of each of the microbial strain was suspended in 2 ml of respective broth solution and incubated overnight at 37^oC (for bacteria)and 30^0C (for fungi).To screen for antimicrobial activity, sterile agar plates were used according to the disc diffusion assay. The contents of media (15 ml) were poured into a sterile clean and dry Petri plates ,Then allowed the media to sterile down. A bent glass (L-rod) was used for spreading diluted culture on the plates. The antimicrobial activity was applied by using Nutrient agar medium. Discs were made by No.1 filter paper (6 mm) and the disc was dipped with 1mg/ml sample test solution and ampicillin was the standard reference antibiotics. After impregnated disc were placed on the microorganism inoculated medium and then plates were incubated in the upright position at 37 ºC for 24h. The plates were periodically checked for microorganism growth after the incubation period and the consequential zones of growth inhibition were accurately measured and expressed in millimeters. Assays were run in triplicates and mean values were tabulated.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of the Petroleum ether, Aqueous , Ethyl acetate and methanol crude extracts of *Actinodaphne lanata* leaf revealed the presence of Phenols, Terpenoids and steroids. Tannins, Saponins and Alkaloids in methanol extracts. Glycosides in ethyl acetate extract, Cardiac glycosides flavonoids, terpenes and reducing sugar in methanol extract. From the result, methanol extracted more of the bioactive constituents. The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators[19].Flavonoids and other phenolic compounds are potent water-soluble antioxidants and free-radical scavengers that prevent oxidative cell damage and exhibit strong anti-cancer activity [20-21].*Actinodaphne* plants of the family Lauraceae have been reported to produce isoquinoline alkaloids (aporphines, oxoaporphines) and lactones[22-23]reported aqueous and methanol extracts are suitable for extraction of secondary metabolities than the alcohol, chloroform, petroleum ether, diethyl ether, acetate and hexane solvents for both *Thespesia* and *Tridax* species Terpenoids. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer[24-25].The result of the preliminary phytochemical screening of the Petroleum ether , ethyl acetate and methanol and Aqueous extracts of *Actinodaphne lanata* dried leaves has been summarized in Table 1.

Antibacterial activities of different extract of *A. lanata* leaf.

Antibacterial activity were tested using different extracts of *A.lanata* leaf. The higher zone of inhibition was noted against *Bacillus subtilis* (26.8±0.66) followed by *Staphylococcus aureus* (24.4±1.14),*Streptococcus pygenes* (22.2±0.19), *Pseudomonas aeruginosa* (20.2±0.15), *Klebsiella pneumonia* (19.4±1.66) and *E.coli* (17.4±1.72),when methanol extract was used. Moderate zone of inhibition was noted *Streptococcus pygenes* (21.3±1.89) and *Klebsiella pneumonia* (19.2±1.05), when ethyl acetic was used(Table.2) .The lowest zone was

recorded in *E.coli* (11.1±1.47),when petroleum ether extract was used. All extract showed antibacterial activity but not the same level. Comparative antibacterial sensitivity testing results of the Petroleum ether, ethyl acetate , Aqueous and methanol extracts of *Actinodaphne lanata* leaf against bacteria isolates are shown in Table.2.

The methanol extract of the leaves of *Lawsonia inermis* showed significant antibacterial activity, comparable to *Cipro flaxacin* against the Gram-negative microorganisms with special reference to *E.coli, Vibrio cholerae* and Shigella species[26].The methanol and acetone extract of *Alstonia scholaris (*stem bark), *Achyranthus aspera* (whole plant) *Moring aoleifera* (leaves), *Tinospora cordifolia* (stem), and *Enicostemahyas opifolium* (stem) were screened for their antibacterial activity using the agar diffusion method[27]. *Ocimum gratissimum, Aegle marmelos, Adhatoda vasica* have been tested for antimicrobial activity on five different human clinical pathogens viz. *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Escherichia coli* and *Klebsiella pneumonia*[28].Good antimicrobial activity was seen in the methanolic extract of *Byrsonimaverbascifolia*[29]. The antimicrobial activities of *Cynaras colymu*s L. leaf and stem extracts were tested against 15 microbial species and the leaf extract was found to be the most effective, followed by stem extracts[30]. The n-hexane, ethyl acetate, n-butanol, methanol and water fractions of sorghum (*Sorghum bicolor* Moench) have been tested for their antimicrobial activity against *Staphylococcus aureu*s, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Candida albicans* and *Bacillus subtilis*, wherein the methanol extract showed high levels of antimicrobial activity than the other fractions[31].

Antifungal activity of different extract of *A. lanata* leaf.

Antifungal activity were tested using petroleum ether, ethyl acetate, methonal and aqueous extracts of *A.lanata* leaf. The higher zone of inhibition was noted against *Aspergillus flavus* (16.08±0.31), followed by *Fusarium oxysporum* (13.3±0.21), *Candida albicans* (11.4±0.21) and *A.niger* (10.3±0.7).when methanol extract was used. Moderate zone of inhibition was noted *A.*flavus (12.95±0.43) when petroleum ether extract was used, Table.3). The lowest zone was recorded in *Aspergillus flavus* (4.75±0.51) when aqueous extract was used. All extract showed antifungal activity but not the same level. Antifungal activity of standard antibiotic was presented in Table.3. The antimicrobial activity from ethanol leaf extract of *Catharanthus roseus from* Saudi Arabia was investigated against some human pathogenic microorganisms (*Staphylococcus aureus* and *E.coli*) as well as pathogenic fungi *Candida albicans*[32].Similar result was obtained while studying the antimicrobial activity of *Bacopa monnieri*. Disc diffusion method was used to evaluate the zone of inhibition against the test organisms. Disc diffusion method is used extensively to investigate the antimicrobial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoir containing solution of the substances to be examined[33].As evident from the results the antimicrobial activity of the extracts against test organisms and these finding correlate with the observation of various screening of medicinal plants for antimicrobial activity[34-35].

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Table1: Summary of phytochemical result of *Actinodaphne lanata* leaf Extracts.

Phytochemical constituents	**Leaves**			
	PET	**EA**	**MeOH**	**Aqueous**
Tannins	-	-	+	-
Saponins	+	-	++	++
Alkaloids	-	-	+	-
Glycosides	-	+	-	-
Reducing sugar	-	-	-	-
Flavonoids	-	-	+	+
Triterpenoids	+	+	+	-
Steroids	+	+	+	++
Terpenoids	+	+	++	++
Phenol	-	-	++	-
Cardio glycosides	-	-	+	-
Resin	+	+	+	-

Key:+ =Present,- Absent

Values are mean ±SD(n=3);Mean values followed by different superscripts in a column are significantly different($p<0.05$)according to Duncan's multiple range tests (DMRT).

Table 2. Antibacterial activity of leaf extract of *A. lanata.*

S.No	Bacterial pathogens	Zone of inhibition (mm)				
		Ampicillin	**Petroleum ether**	**Ethyl acetate**	**Methanol**	**Aqueous**
1	*Streptococcus pygenes*	33.1±1.21	13.0±1.04	21.3±1.89	22.2±0.19	6.3±0.80
2	*Staphylococcus aureus*	29.2±1.03	12.4±0.70	16.7±1.18	24.4±1.14	5.6±1.92
3	*Bacillus subtilis*	28.6±1.67	11.6±0.88	16.1±1.35	26.8±0.66	4.8±0.91
4	*Klebsiella pneumonia*	27.7±1.12	12.1±1.26	19.2±1.05	19.4±1.66	6.9±0.95
5	*E.coli*	24.3±1.28	11.1±1.47	13.4±0.62	17.4±1.72	6.1±1.42
6	*Pseudomonas aeruginosa*	23.1±1.22	12.3±1.19	14.9±0.14	20.2±0.15	3.7±1.64

Values are mean ±SD(n=3);Mean values followed by different superscripts in a column are significantly different($p<0.05$)according to Duncan's multiple range tests (DMRT).

Table.3 Antifungal activities of leaf extract of *A. lanata*

S.No	Fungal pathogens	Zone of inhibition (mm)				
		Tetracycline	**Petroleum ether**	**Ethyl acetate**	**Methanol**	**Aqueous**
1	*Aspergillus niger*	16.23±0.75	9.21±0.31	9.32±0.21	10.3±0.7	6.12±0.61
2	*A. flavus*	19.12±1.23	12.95±0.43	11.32±0.11	16.08±0.31	4.75±0.51
3	*Fusarium oxysporum*	14.13±0.35	10.21±0.61	7.21±0.32	13.3±0.21	8.75±0.13
4	*Candida albicans*	19.72±0.24	7.42±0.64	9.12±0.50	11.4±0.21	9.42±0.42

Values are mean ±SD(n=3);Mean values followed by different superscripts in a column are significantly different($p<0.05$)according to Duncan's multiple range tests (DMRT).