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Phytochemical and Antimicrobial Studies of Leaf and Bark Extracts of *Holigarna arnottiana* Hook f.

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One of the poisonous plants of Western Ghats is *Holigarna arnottiana* Hook f. may cause contact dermatitis and skin irritations. The phytotoxic chemicals present in this plant can be isolated and used for various commercial products. A phytochemical analysis showed the presence of alkaloids, steroids, tannins, and phenolic compounds, flavonoids, steroids, resins, fatty acids, gums which are capable of producing definite physiological action on body. These compounds may be responsible for the antibacterial activities of the leaf and bark extracts.The antioxidant activity also showed promising results. Of the four solvents used methanolic extract showed very promising antibacterial activity against the test strains.

Key words: *Holigarna arnottiana,* leaf, bark extract, antibacterial, phytochemical studies.

Owing to the over usage of chemicals as fertilizers and pesticides the equilibrium between abiotic and biotic world is depleting day by day. Hence human interest is concentrated on plant and plant products. The only remedy to this dreadful situation is to concentrate on our plant world. India has been identified as one of the top 12 mega-diversity countries of the world. Among the 18 hot spots recognized in the world, two are in India–Eastern Himalayas and Western Ghats[1]. The Western Ghats, which is one of the nine biogeographic regions of India, possess various types of tropical forests, ranging from wet evergreen to dry deciduous. Nearly 63% of the tree species of the low and the medium elevation evergreen forests of Western Ghats are endemic[2].This high level of diversity and endemism in the Western Ghats has conferred on them the hot spot status. Of the 62 endemic tree species found in Western Ghats, the *Holigarna arnottiana* Hook.f. belongs to one of them. This indicates that these species have high ecological amplitude and niche overlap[3].

The phytochemicals are the prime and bioactive compounds of plants that are responsible for the extended biological properties. . Innovative approaches to explore the biological properties exhibited by phytochemical compounds present in the plant extracts would be helpful to reach the market and economy to a considerable extent. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenolic compounds, flavonoids, steroids, resins, fatty acids, gums which are capable of producing definite physiological action on body. Therefore, it is a new challenge to seek for the *in vitro* antimicrobial activity of natural compounds

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such as polyphenols from these ethnomedicinal plants on pathogenic bacteria. Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavonols, flavanones, flavones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, aurones, etc. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. In addition to their individual effects, antioxidants interact in synergistic ways and have sparing effect in which one may protect another against oxidative destruction.

Currently, the research of natural antioxidants like alternative sources of synthesis antioxidants was emerged and the exploitation of the various secondary metabolites of the plant was highlighted in recent years. Thus, the phenolic compounds in particular the flavonoids have drawn attention as a potential source of bioactive molecules. There is lack of scientific studies on these selected plants especially antimicrobial studies[4.] The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants[5]. Screening of medicinal plants for antimicrobial and elementological activities are important for finding potential new compounds for therapeutic use.

The present study is concentrated on one of the poisonous plant and "untouched" plant growing in Western ghats, *Holigarna arnottiana* or Charu. People are afraid of to come in contact with this plant as the contact itself may cause skin irritation .There are a large number of myths behind the history of Charu. In this study it is impossible to go through such myths and beliefs. Anyway, by one way or other Charu is remaining as an "untouched" specimen to the scientific world. *Holigarna arnottiana* belongs to the family Anacardiaceae (Cheru in Malayalam, Vanabhallaka in Sanskrit and Holgery in Hindi) endemic to Western Ghats from Central and South Sahyadris. It is generally classified in Ayurveda under the category of toxic plants. There may be a lot of economically important principles present in the plant, it is to be studied scientifically and used systematically. That may be a great step towards modern pharmaceutical fields. In this attempt we are trying to reveal the phytochemical, quantitative analysis of biomolecules and antimicrobial potentialities of plant against some important pathogenic bacteria.

MATERIAL AND METHODS

The plant material collected from Thiruvanathapuram was identified with taxonomic methods and had been documented at the Herbarium of Botany Department ,University College Thiruvananthapuram (Voucher No.11358).

Preparation of Extracts

The phytochemicals present in the leaf and bark of the plant material was extracted by the distillation method using Soxhlet apparatus. Different solvent systems were used for the separation of chemicals according to the polarity (Hexane, Benzene, Ethanol and Methanol). About 500g of leaf and bark were weighed and shade dried for one week. The dried materials were powdered and 50 g of each powder sample was packed in a thimble and kept in Soxhlet apparatus. Each of the solvent was taken separately for the extraction and the powdered material was siphoned by three times. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point of each solvent. The extract was concentrated to dryness and the residues were transferred to a preweighed sample bottle and were stored in a desiccator for further studies[6].

Phytochemical Screening of Plant Different Extracts

Different biochemical parameters like reducing sugars, flavonoids, steroids, terpenoids, tannins, alkaloids, coumarins, saponins, anthraquinones and glycosides were qualitatively estimated[7].

Test for reducing sugars (Fehling's Test)

The aqueous ethanol extract was added

to boiling Fehling solution in a test tube. A brick red colour indicates the presence of reducing sugars.

Test for Flavonoids (Shinoda test)

Take 1 ml of the extract and add a few magnesium turnings, followed by the addition of con. HCl drop by drop. Development of pink colour indicates the presence of flavonoids.

Liebermann- Burchard test for Steroids and Terpenoids

Take 2ml of extract, dry and dissolve in chloroform. Add a few drops of acetic anhydride and con. H_2SO_4 and keep undisturbed for few minutes. Formation of green colour indicates the presence of steroids, while pink colour indicates the presence of terpenoids.

Test for Tannins

To 1ml of the extract, add two drops of 5% $FeCl_3$. Presence of dirty green precipitate indicates the presence of tannin.

Test for coumarins

1ml of the extract was dissolved in methnol followed by the addition of a few drops of alcoholic NaOH. Con. HCl was added through the sides of the test tubes which shows the appearance and disappearance of yellow colour, indicates the presence of coumarians .

Test for Alkaloids

5 ml of the extract material was warmed with 10ml of 2% H_2SO_4 for two minutes and filtered. Treat 1ml of its aliquot with a few drops of Dragendorff's reagent. Presence of orange brown precipitate indicates the presence of alkaloids.

Test for Saponins

5ml of the extract was shaken with 5ml of distilled water and was heated to the boiling point. Frothing indicates the presence of saponin.

Test for Anthraquinones

To 0.5g of powdered material add 10ml of 1%HCl and boiled for five minutes. Filter the sample and allowed to cool. Partition the cool filtrate against equal volume of chloroform. Carefully transfer the chloroform layer into clean test tubes. Shake with equal volume of 10% ammonia solution and allow the layer to separate. Presence of delicate rose pink colour indicates the presence of combined anthraquinones.

Test for Glycosides (Keller-Killiani Test)

To 0.5g of extract diluted to 5ml with distilled water and add 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with one ml of con.H_2SO_4. A brown ring at the interface indicates the presence of glycosides.

Quantitative Test for Leaf and Bark Tissue

Determination of Moisture

5g of the material was taken in a preweighed petridish. The petridish was placed without lid into an oven at 110°C for three hours. The Petridish was taken out and closed immediately with a lid. The dish was cooled in a desiccator and weighed. The amount of moisture of the material was calculated from the difference in weight[8].

Total Carbohydrate

Estimation of total carbohydrates was done by the method of Roe *et al*[9]. Weighed amount of fresh tissue was homogenized with distilled water .The homogenate was filtered using a two layered cheesecloth. The filtrate was then centrifuged at 10,000g for 15 min. The supernatant was collected and the volume was made up to 25 ml using distilled water. An aliquot of sample was pipetted out and 4ml Anthrone reagent was added. It was then kept in a boiling water bath for 10 min. The tubes were cooled and the absorbance was measured at 530nm. The amount of total carbohydrates present was determined using the standard graph of glucose.

Total Cellulose

The weighed amount of freshly collected plant materials were taken in a test tube containing acetic – nitric reagent and mixed in a vortex mixer. They were placed in a water bath at 100^0 C for 30 min ,were cooled and centrifuged at 10000 g for 15 sec. The supernatant discarded and washed the residue with distilled water.10 ml of 67% sulphuric acid was added to the residue and allowed to stand for 1 hr. 1ml the above solution was diluted to 100ml followed by the addition of 10ml of anthrone reagent and mixed well. The tubes were heated in a boiling water bath for 10 min. Tubes were cooled and the absorbance was measured at 630 nm against a suitable blank. The amount of total Cellulose present was calculated using the standard graph of cellulose[10].

Total Protein Estimation

The total protein was estimated as per

Peterson[11], Fresh leaves and bark were collected in the morning were washed in running tap water followed by sterile distilled water and was chopped to randomize. 1 gm randomized sample and was homogenized with phosphate buffer (pH 7) in a pre chilled mortar and pestle (acid washed sand was used for thorough grinding). The extract was filtered and centrifuged at 5000 rpm for 10 min. The supernatant was dispensed to a measuring jar. The residue was again centrifuged at 3000 rpm for 5 min and all the supernatants were pooled together and made to a known volume using the same buffer.

From this 5 ml aliquot was drawn and 2ml 100% TCA added. It was then vortexed, kept for 30 min. and Centrifuged at 5000 rpm for 10 min. to collect the protein pellet. The chlorophyll molecules were removed by washing the pellet with 100% acetone and centrifuged. The starch molecules were eliminated by repeated centrifugation using 5% PCA. The pellet was dissolved in 5ml of 0.1N NaOH in a boiling water bath and was further centrifuged to remove the debris.

Different aliquots were pipetted out, and made up to 1mL using distilled water. To this was added 5ml Reagent C and 0.5ml Folin-ciocalteau reagent and was kept at 30 min at room temperature. The absorbance was read at 650nm against a reagent blank. The amount of total protein in the leaf was calculated using a standard of BSA.

Total Phenol Estimation

Estimation of total phenols was performed by the method of Mayr *et al.*[12]. Weighed amount of fresh tissue was chopped and put in boiling methanol (80%) for 10 min. and refluxed. The refluxed matter was homogenized. The homogenate was filtered and centrifuged at 10,000rpm for 10 min. The supernatant was collected and the volume was made upto 20 ml using 80%methanol. 0.5ml Folin-Ciocalteau reagent followed by 2 ml 20% sodium carbonate was added to an aliquot of sample and mixed thoroughly. The reaction of phenol with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium produced a blue coloured complex. The tubes were kept in a boiling water bath for one minute, cooled and centrifuged, supernatant was taken and the absorbance was measured at 650 nm. The amount of total phenols present was determined using a standard graph of catechol.

Estimation of Tannin

Tannin present in the samples was estimated by Folin-Denis method. Tannin like compound reduces phosphotungsto molybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to the amount of tannin. The intensity is measured at 700nm.

2g of powdered tissue was taken in a 250ml conical flask and 75ml distilled water was also added. It was then heated gently for 30 minutes and centrifuged at 2000rpm for 20 minutes. The supernatant was collected and made up to 100ml with distilled water. An aliquot of 1ml extract was made up to 75 ml with distilled water. 5ml Folin-Denis reagent and 10ml sodium carbonate was also added. Then the solution was diluted to 100ml with distilled water. The absorbance was recorded spectrophotometrically at 700nm after 30 minutes against a suitable blank. Total amount of tannic acid was calculated from the standard graph of Tannic acid[8].

Anti oxidant activity assay

DPPH(1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity

The H-donor activity of the extract was estimated in this method. Different concentrations of the methanol, ethanol, benzene and hexane extracts of 10- 100 mg/ml was prepared.100 mM of DPPH radical solution in methanol was prepared. 50ml extract of different concentration was added to 2.95ml of DPPH solution and the reaction mixture was incubated at 37°C for 15 minutes. The absorbance was read at 517 nm against positive control which did not contain the extract. Ascorbic acid was used as standard. The assay was carried out in triplicate. A decrease in absorbance of DPPH solution indicates the increased DPPH scavenging activity. The activity is given as percentage DPPH radical scavenging [13].The radical scavenging activity was calculated using the formula,

$$\% \text{ inhibition} = [(Ab-Aa)/Ab] \times 100.$$

Where,

Ab is the absorbance of the blank sample and Aa is the absorbance of the extract. Extract

concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting the inhibition percentage against concentration.

Antibacterial Studies of Different Extracts

The leaf and bark extracts prepared in different solvents by the Soxhlet apparatus were used for the antimicrobial studies. All the glass wares including petriplates, conical flasks, beakers etc. were sterilized by autoclaving at 121°C for 15 minutes. Different bacterial strains were used for anti bacterial studies. They include *Staphylococcus aureus, Bacillus subtilis, E.coli* and *Pseudomonas aeruginosa* and are collected from CEPC, Kollam.

Preparation of peoptone water

Peptone water was prepared by adding 13.6g of peptone in 1000ml distilled water[14].The solution was sterilized using an autoclave at 121°C for 20 minutes. The bacteria from the culture stock are inoculated into the peptone water and were kept 37° C for growth.

Preparation of nutrient agar medium

The nutrient agar medium was prepared by adding 28g of nutrient agar in 1000ml distilled water. For the solidification of the medium 10g of agar was dissolved by placing it in the boiling water bath. Media was autoclaved at 121°C for 15minutes.Required volume of molten medium was poured into the sterile Petri dishes under aseptic condition[14].

Disc Diffusion Method for Antibacterial Studies

Pure cultures of *Staphylococcus aureus, Bacillus subtilus, and E.coli* and *Pseudomonas aeruginosa* from the peptone water is taken with sterile swabs and evenly distributed the bacteria on the nutrient agar plates. Disc diffusion method was used to study the antibacterial effects of the different plant extracts. Discs of 4mm diameter were cut out from Whatman no. 42 filter paper and sterilized by autoclaving. The discs were treated with different concentrations (1mg/ml, 2mg/ml and 3mg/ml) of each extracts. The control disc was prepared by treating with DMSO (Dimethyl sulfoxide) and standard by antibacterial drug, streptomycin[15]. The prepared discs containing the different concentrations of extracts, control disc and standard disc were placed on each petriplates containing pure culture of bacteria. Then it was kept for incubation at 37^0 C for three days.

RESULTS AND DISCUSSION

Phytochemical Screening of different plant extracts

Preliminary phytochemical screening on *Holigarna arnottiana* leaves and bark extract revealed the presence of reducing sugars flavonoids , steroids and anthraquinones in the hexane extract .The benzene extract of leaves showed the presence of terpenoids in addition to the hexane extract while flavonoid and terpenoids were absent in the bark benzene extract.

The study showed that the ethnolic extract of bark contain most of the phytochemicals like reducing sugars flavonoid, terpenoids, tannins, coumarins, alkaloids, saponins, anthraquinone and glycosides except steroids . But steroids and glycosides were absent in the leaves methanol extract.

Flavonoids, steroids, coumarins and glycosides were absent in the leaves ethanolic extract while coumarins was present and alkaloids was absent in the bark ethanolic extract (Table 1)

Quantitative Test for Leaf and Bark Tissue

The result of proximate analysis showed variant concentrations of biochemicals and other content. The moisture content of leaf was found to be 5.80mg/g fresh tissue while in the bark it was only about 3.06 mg/g fresh tissue. Looking at the result obtained from the carbohydrate analysis of leaf had prominent level compared to bark. While analyzing the protein content in the leaf and bark of *Holigarna arnottiana*, the result showed that leaf had highest concentration of protein compared to bark. The result of cellulose analysis showed that leaf had highest concentration than the bark. But in comparative assessment of phenol and tannin content of bark and leaves, it was found that both were higher than those in the leaf tissue (Table2).

Antioxident activity (DPPH Scavenging) of leaves different extract

The antioxidant activity of different extract of *Holigarna arnottiana* Hook.f. examined by comparing it to the activity of known antioxidant such as ascorbic acid. As shown in the Table 3 at 50 mg/ml and above all the extract of leaves showed good scavenging activity comparable to the control compound L-ascorbic acid. At 100 mg/ml the methanol extract reached

Table 1.Phytochemical Screening of Hexane, Benzene, Ethanol and Methanol Extract of Leaf and Bark of *Holigarna arnottiana*

Qualitative test	LEAF				BARK			
	Hexane	Benzene	Ethanol	Methanol	Hexane	Benzene	Ethanol	Methanol
Reducing sugars	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	-	-	+
Steroids	+	+	-	-	+	+	-	-
Terpenoids	-	+	+	+	-	+	+	+
Tannins	-	-	+	+	-	-	+	+
Coumarins	-	-	-	+	-	-	+	+
Alkaloids	-	-	+	+	-	-	-	+
Saponins	-	-	+	+	-	-	+	+
Anthraquinones	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	+

+ = present - = absent

Table 2. Proximate Quantitative Analysis of Various Biochemical Parameters of *Holigarna arnottiana*

Quantitative	LEAF (mg/g FW)	BARK (mg/g FW)
Moisture Content	5.80±0.03	3.06±0.04
Total Carbohydrate	54.21±3.14	52.76±4.21
Total Cellulose	38.20±3.11	32.26±2.26
Total Protein	20.64±1.21	19.43±2.01
Total Phenol	18.45±2.21	21.28±1.81
Total tannin	2.23±1.10	3.14±0.98

a similar magnitude of activity above 80% when compared to standard. The order of free radical scavenging potency for the tested extracts from the test tissues of *Holigarna arnottiana* was as follows: methanol extract > ethanol extract > benzene extract > hexane extract. This perhaps indicates that the free radical scavenging activity of methanol and ethanol extracts of leaf were $\geq$70% .The hexane extract was found to be poor when compared to other extracts. Both methanol and ethanol extracts of *Holigarna arnottiana* exhibited equal potency.

The methanol extract was able to reduce the stable free radical DPPH to the yellow coloured Diphenyl picryl hydrazine with an IC_{50} of 12.41+0.28 mg/ml exhibiting better activity as compared to Ascorbic acid (8.53±0.18 mg/ml) while the ethnolic, benzene and hexane extract had IC_{50} values of 17.62±0.41mg/ml, 23.29±0.39mg/ml and 34.81± 0.26mg/ml respectively (Table 4).

Table 3. Effect of Different Extracts from *Holigarna arnottiana* and positive control (L-Ascorbic acid) on the *in vitro* free radical Scavenging Activity

Concentration (mg/ml)	Hexane (%inhibition)	Benzene (%inhibition)	Ethanol (%inhibition)	Methanol (%inhibition)	L-Ascorbic acid (%inhibition)
10	44.12±0.32	46.41±0.39	48.24±0.13	49.11±0.31	51.12±0.35
20	46.18±0.17	49.71±0.51	51.18±0.26	56.61±0.45	58.42±0.45
30	49.18±0.47	53.32±0.63	55.92±0.38	59.32±0.24	60.81±0.41
40	52.53±0.35	55.39±0.49	58.48±0.47	61.34±0.48	63.53±0.52
50	56.32±0.36	59.41±0.64	62.18±0.45	66.44±0.36	67.92±0.25
60	59.91±0.28	61.16±0.29	67.66±0.54	69.63±0.46	70.18±0.31
70	62.24±0.46	65.36±0.27	69.73±0.38	70.42±0.57	72.21±0.16
80	64.71±0.37	68.49±0.46	71.28±0.47	74.42±0.71	76.22±0.24
90	67.21±0.52	70.03±0.54	75.57±0.64	78.61±0.49	81.17±0.36
100	69.08±0.36	74.13±0.29	78.51±0.61	80.41±0.81	84.38±0.69

Antibacterial Activity of Different Extracts

The preliminary antibacterial screening, indicated hexane extract, benzene extract, ethanol extract and methanol extract of *Holigarna arnottiana* to be effective against the test organism. The result of antibacterial activity of leaves and barks extract of *Holigarna arnottiana* against bacteria *viz Staphylococcus aureus, Bacillus subtilis, E.coli* and *Pseudomonas aeruginosa* are shown in the Table 5-12. The inhibition zone diameter (IZD) of different solvent extract against different gram positive and gram negative bacteria ranging from 3-22mm.

Table 4. Effect of Different Extracts from *Holigarna arnottiana* and positive control (L-Ascorbic acid) on the *in vitro* free radical Scavenging Activity (IC_{50} Values)

Sample	DPPH IC 50 (mg /ml)
Hexane	34.81 ±0.26
Benzene	23.27± 0.39
Ethanol	17.62 ±0.41
Methanol	12.41 ±0.28
L-Ascorbic Acid	8.53 ±0.18

The result showed that methanolic extract of leaf showed IZD of 22mm against *Staphylococcus aureus* at a concentration 3mg/ml. The ethanolic, benzene and hexane leaf extract showed 18mm, 21mm and 16mm of IZD respectively against the same bacterium at concentration of 3mg/ml (Table 5-8).

The methanolic extract of leaf showed less IZD for *E. coli* at 1mg /ml (Table 5). The

Table 5. Antibacterial activity of Methanol Leaf Extract of *Holigarna arnottiana*

Methanol Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
Control(C)	0	0	0	0
1	7	6	4	7
2	15	13	9	11
3	22	21	17	19
Standard(S)Streptomycin	23	19	17	18

Table 6. Antibacterial activity of Ethanol Leaf Extract of *Holigarna arnottiana*

Ethanol Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
C	0	0	0	0
1	9	4	6	5
2	11	8	10	9
3	18	11	17	16
Standard(S)Streptomycin	19	15	21	20

Table 7. Antibacterial activity of Benzene Leaf Extract of *Holigarna arnottiana*

Benzene Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
C	0	0	0	0
1	10	4	3	6
2	12	9	6	10
3	21	18	14	17
Standard(S)Streptomycin	18	21	21	19

Table 8. Antibacterial activity of Hexane Leaf Extract of *Holigarna arnottiana*

Hexane Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
Control (C)	0	0	0	0
1	7	6	5	7
2	11	9	7	9
3	16	15	10	15
Standard(S)Streptomycin	20	17	14	17

Table 9. Antibacterial activity of Methanol Bark Extract of *Holigarna arnottiana*

Methanol Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
Control (C)	0	0	0	0
1	8	6	5	4
2	12	13	11	9
3	19	22	15	16
Standard(S)Streptomycin	20	21	16	22

Table 10. Antibacterial activity of Ethanol Bark Extract of *Holigarna arnottiana*

Ethanol Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
Control (C)	0	0	0	0
1	4	5	7	8
2	7	10	9	11
3	10	18	12	15
Standard(S)Streptomycin	13	17	14	16

Table 11. Antibacterial activity of Benzene Bark Extract of *Holigarna arnottiana*

Benzene Extract	*Staphylococcus aureus* (mm)	*Bacillus subtilis*	*E. coli*	*Pseudomonas aeruginosa*
Control (C)	0	0	0	0
1	3	5	7	3
2	7	9	10	6
3	11	13	15	10
Standard(S)Streptomycin	14	16	19	15

Table 12. Antibacterial activity of Hexanel Bark Extract of *Holigarna arnottiana*

Hexane Extract	*Staphylococcus aureus*(mm)	*Bacillus subtilis aeruginosa*	*E.coli*	*Pseudomonas aaeruginosa*
Control (C)	0	0	0	0
1	3	7	4	3
2	7	11	9	6
3	13	17	15	10
Standard(S)Streptomycin	15	16	14	13

ethanolic extract were more effective against *Staphylococcus aureus* of IZD 18mm at 3mg/ml and least was against *Bacillus subtilus* (Table 6) ;Whereas the benzene extract was effective against *Staphylococcus aureus* of 21mm IZD and did not show significant result against *E. coli* (Table 7). According to the result given in the Table 8, the hexane extract had less antibacterial activity against the selected strains of bacteria.

Antibacterial Activity of Bark Different Extracts

The antibacterial activity of the bark extract is given in the Table 9 to12. As can clearly seen from this table, the extracts provided from bark of *Holigarna arnottiana* Hook.f. were found to be effective against the *Staphylococcus aureus*, *Bacillus subtilis, E.coli* and *Pseudomonas aeruginosa* showing inhibition zone ranging of 3-22mm.

Methanolic extract of bark showed significant antibacterial activity against the selected bacteria. The methanolic and ethnolic extract had highest antibacterial activity against *Bacillus subtilis* and least against *Pseudomonas aeruginosa* which is higher than the standard antibiotic streptomycin and least against *Pseudomonas aeruginosa* (Table 9,10). The benzene extract inhibit the growth of *E. coli* upto 15 mm at a concentration of 3 mg/ml while it inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* only up to 3mm (Table 11). The hexane extract showed highest activity against *Bacillus subtilis* and is significant where the streptomycin inhibit the growth which is lesser than the extract (Table 12).

Correlation of the present investigations

Phytochemical studies

On the phytochemical screening of the leaf and bark extract showed the presence of reducing sugars, flavonoids, steroids, terpenoids, tannins, alkaloids, coumarins, saponins, anthraquinones and glycosides, in various solvents like benzene, hexane, methanol and ethanol. They show considerable differentiation with respect to the solvent also. This kind of phytochemicals were previously reported from the members of Anacardiaceae by various authors[16,17,18,19]. The present study on *Holigarna arnottiana* also revealed the presence of such compounds in various quantities which show their chemical relationship with the other members of the family. The Anacardiaceae members produce allergic reactions like dermetitis due to the presence of phytoconstituents like alkyl catechols, phenols, quinols and resorcinol[20]. The present study also correlates the contact dermatitis that occurs may be due to phytochemicals found in the bark and leaf (Table 1 and 2).

Besides this the proxamate quantitative analysis of various biochemical parameters of *Holigarna arnottiana* Hook.f. will also show direct relationship with that of other members of the family Anacardiaceae[17,22,23]. This also indicates their direct relationship to the other members with respect to the poisonous activities such as contact dermatitis. The present study reveals the concentration of total phenol, total protein and total tannin percentage much higher than that of the related members which may be the reason for the skin allergies and contact dermatitis in general.

Antioxidant Activity

The antioxidant acitivity of *Holigarna arnottiana* methanol extract was detected as 80.41% and it was almost same as that of the standard L-Ascorbic acid with 84.38% (Table 3). The order of the free radical scavenging potency was methanol extract > ethanol extract > benzene extract > hexane extract. By the present study it was identified that the most pronounced effect was that of methanol extract and least in the case of hexane. The methanol extract was able to reduce the stable free radical DPPH to yellow colour compound with and IC_{50} of 12.41 ± 0.28µg/ml exhibiting better activity as compared to standard Ascorbic acid (Table 4).

Antibacterial Studies

The antibacterial studies conducted presently with four extract on to separate samples, leaves and bark showed wide variation with respect to their effects (Table 5-12). The inhibition zone diameter of different solvent extract against different Gram positive and Gram negative bacteria ranging from 3-12mm.

From the present investigation it was proved that the methanolic leaf extract is very efficent to control all the four strains of bacteria in 3mg/ml concentration, which was more efficient than the standard Streptomycin against

the *Bacillus subtilis* and *Pseudomonas aeruginosa* with 21mm and 19mm IZD were as the standard showed 19mm and 18mm IZD. The inhibiton zone diameter against the cultures of *Staphylococcus aureus* and *E.coli* were 22mm and 17mm and that of standard was 23mm and 17mm respectively (Table 5). The ethanolic and benzene leaf extract also showed promising antimicrobial activities against the test strains but hexane leaf extract with comparatively poor IZD (Table 6-8). The methanolic and ethanolic extract of bark had higher antibacterial activities against *Bacillus subtilis* and is higher than the standard streptomycin (Table 9 and 10). The other extracts *ie.* benzene and hexane also inhibited the bacterial growth but in a reduced rate (Table 11 and 12).

CONCLUSION

Based on the present studies it was made clear that *Holigarna arnottiana* Hook.f. was a promising herbal remedy to microbial growth. The most pronounced antimicrobial activity was shown by methanolic extracts of leaves and bark, with the most efficiency in the case of leaf extract. This preparation alone or in combination with ethanolic or other solvents may act as very promising antibacterial agents to the pathogenic bacteria.

REFERENCES

1. Myers, N., *The Environment.*, 1988; **8**: 1–20.
2. Ramesh, B. R., Pascal, J. P. and De Franceschi, P., in *Proc. of the Rare, Endangered and Endemic Plants of the Western Ghats* (ed. Karunakaran, C. K.), K.F.D. (Wildlife Wing), Trivandrum, 1991; 20-29.
3. Nayar, M. P., *Hot Spots of Endemic Plants of India, Nepal and Bhutan,* The Director, TBGRI, Trivandrum, 1996; 252.
4. Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L., Antibacterial screening of some Peruvian medicinal plants used in Callería Dt, *J. Ethnopharmacol.,* 2005; **99**: 309-312.
5. Erdogrul,O.T., Antibacterial activities of some plant extracts used in folk medicine, *Pharmaceutical Biol.,* 2002; **40**: 269-273.
6. Harborne, J.B., *Phytochemical methods*, Chapman and Hall, London, 1973; 1-7.
7. Harborne, J.B., *Phytochemical methods*, Chapman and Hall,London, 1998; 278-281.
8. Sadasivam,S. and Balasubramanian, T., *In. to Practical manual in Biochemistry*, TNAU, Coimbatore, 1987; **14**: 27.
9. Roe, J.H., *Determination of food carbohydrates*. D.A.T. Sathgate, Uni.Cambr., England 1965; 258.
10. Updegroff, D.M., *Anal Biochem*: Academic Press, New York 1969; **32**: 420.
11. Peterson,G.I., Simplification of protein array method of Lowry et al., which is more generally applicable. *Anal.Biochem.,*1979; **83**: 346-356.
12. Mayr, V.D., Trutter, C., Santos-Dudga,H., Baner and Senchitt,W., Developmental changes in the phenol concentrations of golden delicious apple fruits and leaves. *J. Phyto.Chem*., 1995; **38**(5): 1151-1155.
13. Al-saikhan , Howard, M.S. and Miller, J.C., Antioxident activity of total phenolic in different genotype of potato, *J. Food Sci*., 1995; **60**(2): 341-343.
14. Pelezcar,M.J. , *Microbiological methods by the society of American Bacteriologist*, Mc Graw Hill Book Company, 1975; 42.
15. Collins, C.H. & Patricia, M.L. Microbiological Methodes, Butterworths, London, 1976; .224.
16. Chryssavgi, P.,Vassiliki,M.,Athanasios, Kibouris and Michael, K., Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts. *Food Chemistry,* 2008; **2**: 223-227.
17. Prajapati, N.D., Purohit.S.S., Sharma,A.K. and Kumar, T., *A Hand book of Medicinal Plants*. 3rd Edition. Agrobios Hindustan Printing Press, Jodhpur 2006.
18. Mossal Jaber S, Rafatullah S., Ahmed M. Galal and Al-Yahya M.A., Pharmacological studies of *Rhus retinorrhaea. Int. J. of Pharm,* 1995; **4**: 214-216.
19. Prakasa Rao, N.S., Ramachandra Row,L. and Brown, R.T., *Phytochem*., 1973; **12**: 671-681.
20. Kubo, I., Ochi, M., Vieira, P.C. and Komatsu, S., Antitumor agents from the cashew *Anacardium occidentale* apple juice, *J. Agri. Food Chem*., 1993; **41**: 1012-1015.
21. Ohler, J.G., Cashew; Koninklijk Instituut voor de Tropen: Amsterdam, Netherlands, 1979; 260.
22. Cowan, M.M., Plants products as antimicrobial agents, *Clin. Microbiol. Rev*., 1999; **12**: 564-582.
23. Aiyelaagbe, O.O. and Paul, M., Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan,Oyo State, *Plant science research*, 2009; **2**(1): 11-13.