Evaluation of phytochemical and antimicrobial properties of leaf extracts of *Ardisia solanacea* Roxb. collected from Pathiramanal Island, Alappuzha District, Kerala State, India

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Abstract: *Ardisia solanacea* is a large evergreen shrub growing up to a height of 1.5 to 4 meter tall and native to Bangladesh, China, India, Malaya, Nepal, Sri Lanka and Pakistan. *A. solanacea* is a one of the important herb reported to contain an array of phytochemicals and is widely used as food and in traditional medicine. The objective of this study was to analyze the phytochemicals present in the dried leaf samples and to evaluate its antibacterial and antifungal properties. For the evaluation of antibacterial and antifungal properties, agar well diffusion method was used. Phytosterols, phenols and tannins were present in all solvent extracts. Higher antibacterial activity was observed in aqueous hot leaf extract against *Staphylococcus epidermidis* (13 mm), *Escherichia coli* (12 mm) and *Xanthomonas campestris* (12 mm) and methanol cold extract showed the highest activity against *Bacillus cereus* (8 mm), *Klebsiella pneumoniae* (9 mm), *Pseudomonas aeruginosa* (10 mm) and *Vibrio parahaemolyticus* (8mm). Only methanol hot extract showed antifungal properties against *Aspergillus fumigatus*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Trichophyton rubrum*.

Keywords: antibacterial activity, *Ardisia solanacea*, *Aspergillus fumigatus*, pathogenic bacteria, phytochemicals, Staphylococcus *epidermidis*.

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Introduction

Ardisia solanacea is a species of the genus *Ardisia* are found throughout tropical and subtropical regions of the world. These species has been used as ornamental plants, medicines and food as well. *Ardisia* group is the famous herbs in China, which have been used as medicinal plants for more than 900 years. *A. solanacea* has credited with different local names in various regions of India. In the regions of Kerala, where it is being called Kakkanjara. It is widely used in the treatment of fits, eye pain, and also leaf used as vegetable, analgesic and cattle feed (Chandran et al. 2015). This plant is traditionally important in the treatment of fever, diarrhoea, pains, bacterial infections and liver disorders (Kobayashi and de Mejia 2005). *Ardisia* species has several biologically active phytochemicals including saponin, coumarins and quinines. *A. Solanacea* leaf was collected from Pathiramanal island, Kuttanad (9° 17′ to 9° 40′ N latitude and 76° 19′ to 76° 33′ E longitude), which forms a part of the Vembanad wetland system, one of the Ramsar sites in Kerala, India. It is located on the south-western end of Indian peninsula. *A. solanacea* is reported from different parts of the Western Ghats in India and also in Indo- Malesia and West China (Sasidharan 2004; Chandran et al. 2015).

Nearly 500 species of the genus *Ardisia* are recorded throughout the tropical and subtropical parts of the world. They are used as medicinal and ornamental plants, apart from forming a wild fruit resource for the natives. *Ardisia* spp. has several biologically active phytochemicals, including saponins, coumarins and quinines, and is a rich source of biologically potent compounds, such as bergin and ardisin (Kobayashi and de Mejia 2005). The plants having phytochemicals possess medicinal value that can have physiological and pharmacological actions on human body (Hossain et al. 2016). The plant is applied in the treatment of diarrhea, dysmenorrhea, gout, mental disorder, rheumatic arthritis, skin sore and vertigo. Roots have antibacterial activity. Other species of the Ardisia have been reported for their cytotoxic, thrombolytic and antioxidant properties (Khatun et al. 2013). Because of the reported uses of Ardisia plants in traditional medicine, we have attempted to find out the phytochemicals present in the plant, its antibacterial and antifungal property of *Ardisia solanacea*.

Materials and Methods

Collection and identification of plant

The plant, *Ardisia solanacea* was collected from Pathiramanal Island Alappuzha, Kerala, India. This plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre, P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India and the voucher specimen (Collection number 7601) was deposited in the Herbarium of Environmental Resources Research centre (ERRC), Thiruvananthapuram, Kerala. The plant leaves were washed several times with water, shade dried and then pulverized to coarse powder in an electric grinder. The powder was then stored in air tight bottles for further studies.

Chemicals

The solvents (hexane, chloroform, dichloromethane, ethyl acetate and methanol) used for the extraction process were of analytical grade, procured from the SD Fine Chemicals, Mumbai, India. Hexane, chloroform, dichloromethane, ethyl acetate, acetic acid, methanol, acetone, H_2SO_4, $FeCl_3$, HCl, HNO_3, NaOH, KOH, Mueller Hinton agar, Potato dextrose agar, Nutrient agar and other chemicals were procured from Hi-Media Laboratories Private Limited, Mumbai, India.

Fluorescence analysis

Fluorescence analysis is an important phenomenon exhibited by various chemical constituents present in plant substances. If the material themselves are not fluorescent they must often be changed into fluorescent derivatives by reagents. Thus some crude drugs are often assessed qualitatively in this way, and it is significant parameter of pharmacological evaluation (Akhtara *et al.,* 2018). The fluorescence characteristics of the powdered leaf were studied under long UV (315 to 400 nm), short UV (100 to 280 nm) and visible lights (390 to 700nm) after treating with different chemicals such as acetic acid, methanol, chloroform, acetone, 5% H_2SO_4, 5% $FeCl_3$, 1N HCl, 50% HNO_3, 1N NaOH, 5% KOH and H_2O.

Determination of percentage extractive

5 grams of leaf powder was macerated with 50 ml of respective solvents in (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) closed flask for 24 hours and was occasionally shake with 6 hour time intervals and was allowed to stand for 24 hours. After filtration 25 ml of the filtrate evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. Percentage of water soluble extractive value was calculated with reference to the air dried drug. The percent extractive of each solvent extract of *A. solanacea* was calculated by using the formula,

Percent extractive = Weight of dried extract / Weight of dried plant material × 100

Analysis of phytochemical constituents

Analysis of the plant for various phytochemical constituents present was carried out for different solvent (hexane, chloroform, dichloromethane, ethylacetate, methanol, and water) extracts using standard methods described by Sofowara 1993 and Trease and Evans 1989.

Test for Carbohydrates

Molisch's test was performed to detect carbohydrates. Add a few drops alcoholic solution of alpha naphthol to the extracts. Then add about 1 ml of concentrated sulphuric acid along the sides of the test tube. Formation of violet ring at the junction of the liquids indicates the presence of carbohydrates.

Test for alkaloids

Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown coloured precipitate indicates the presence of alkaloids.

Test for cardiac glycoside

Keller-Kelliani test was performed to detect cardiac glycoside. 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates

a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for coumarin glycoside

10% NaOH was added to the extract and chloroform was added for observation of yellow colour, which shows the presence of coumarin.

Test for saponins

Foam test was performed to test the presence of saponins. To 2 ml of extract was added 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for flavonoids

Alkaline reagent test was performed to test the presence of flavonoids. Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colour less on addition of few drops of diluted acid which indicates the presence of flavonoids.

Test for phytosterols

Salkowski test was used to detect phytosterols. To 2 ml of aqueous extract, 2 ml of chloroform and 2 ml of conc.H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for fats and oil

Spot test was performed for fats and oils. This was done by prepared spot on the filter paper with the test solution and oil staining on the filter paper indicates the presence of fixed oil & fats.

Test for phenols and tannins

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicates the presence of phenols and tannins.

Test for proteins

Ninhydrin test was employed to detect the presence of proteins. Crude extract when boiled with 2 ml of 0.2 % solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for terpenoids

1 ml of the extract is treated with Borsche's reagent (2, 4-dinitrophenyl hydrazine in methyl alcohol) and 1ml of 3M HCl. Formation of orange colour indicates the presence of terpenoids.

Preparation of plant extract

The powdered leaf samples (30g) were taken in a conical flask and subjected to cold extraction using methanol and water (250 ml each) for 72 hours. Extracts were filtered using a Whatman No 1 filter paper and was concentrated under reduced pressure at 40ºC using a rotary evaporator (IKA RV 10 digital). For hot extraction the powdered leaf samples were extracted using soxhlet apparatus with methanol and water as solvents. 40g of powdered leaf were extracted using 350 ml of solvent in a soxhlet apparatus for 6 hours. The excess solvent in the extracts was removed by using rotary evaporator. The concentrated samples were weighed, kept in screw capped bottles and stored at room temperature.

Pathogenic Bacterial and Fungal Strains

Nine bacterial strains were used in the present study. Out of the 9 bacterial cultures investigated, two are gram positive bacteria, *Bacillus cereus* (MTCC 430) and *Bacillus subtilis* (MTCC 619), while the remaining 7 are gram negative bacteria. They are *Escherichia coli* (MTCC 729), *Klebsiella pneumoniae* (MTCC 432), *Pseudomonas aeruginosa* (MTCC 4676), *Vibrio cholerae* (MTCC 3904), *Vibrio parahaemolyticus*, *Xanthomonas campestris* and *Staphylococcus epidermidis*. Plant and human pathogenic fungal strains used are *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 873), *Aspergillus fumigatus* (MTCC 343), *Aspergillus tubingensis* (MTCC 2425), *Alternaria alternata* (MTCC 2604), *Cryptococcus gastricus* (MTCC 1715), *Phytophthora infestans*, *Rhizoctonia solani*, *Fusarium oxysporum* (MTCC 284) and *Trichophyton rubrum* (MTCC 296). All these strains were

purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. These bacterial and fungal strains were sub cultured frequently in suitable agar slants and stored at 4°C for further studies.

Preparation of inoculum

The bacterial strains were inoculated in nutrient broth and incubated at 37°C for 24 hours in shaking incubator and this was used for further studies while the fungal strains were inoculated in potato dextrose broth and incubated at 25°C in shaking incubator and used for further studies after 24 hours of incubation.The turbidity of the cultures were adjusted to equal the turbidity of 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. About 100 µl of inoculum of test organism was spread on Mueller Hinton agar plates.

Agar well diffusion method

Agar well-diffusion method was performed to determine the antimicrobial activity (Al-Abd et al., 2015). Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 12 hour old broth culture of respective bacteria and fungi. This process was repeated by streaking the swab two or more times rotating the plates approximately 60° each time to ensure even distribution of inoculum. After inoculation, the plates were allowed to dry at room temperature (20 ±2°C) for 15 minutes in laminar chamber for settling down of inoculum. Wells (6 mm diameter and about 2 cm a part) were made in each of these plates using agar well puncture. 10 µl of extracts were placed on the surface of the bacteria seeded agar plates and it was allowed to diffuse for 5 min. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours for fungal pathogens. The diameter of the inhibition zone (mm) was measured. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Results

Fluorescence analysis

Fluorescence analysis of dried powder of *A. solanacea* was noted under visible light, short UV and long UV which signified their colour characteristics and purity, using the successive solvent extracts like methanol, water, 1N NaOH, 1N HNO_3, 1N HCl, acetic acid, chloroform, acetone, 5% $FeCl_3$, 5% KOH, 5% H_2SO_4, 50% HNO_3. The different colour characteristics

were observed in different chemicals are given in the table 1. The samples of dried powder of *A. solanacea* showed unique colours and it varies from light green to dark green. In certain solvents like methanol, when exposed to long UV, it showed black colour and when exposed to short UV, it showed dark green. In acetic acid, when exposed to short UV, it showed dark green fluorescence. In alcoholic KOH, when exposed to long UV, it showed dark green fluorescence. Similarly, in 5% H_2SO_4 when exposed to short and long UV, it showed dark green and black fluorescence respectively. The presence of unique colours indicated that absence of any adulteration. Fluorescence analysis showed the presence of fluorescent compounds in all the extracts and in the drug powder, which indicated that, the presence of chromophore in this plant. The results of other fluorescence analysis performed are given in table 1.

Chemical/Solve	Fluorescent tut (390 to 700 nm	Short wave (100 to 280 nm	Long wave (315 to 400 n
Powder	Light green	Light green	Dark green
Methanol	Dark green	Dark green	Black
Acetic acid	Dark green	Yellowish gree	Black
Water	Light green	Light green	Black
Chloroform	Dark green	Black	Black
Acetone	Dark green	Dark green	Black
5% $FeCl_3$	Light green	Yellowish gree	Black
1 N NaOH	Dark green	Blackish greer	Black
5% KOH	Dark green	Blackish greer	Black
1 N HCl	Light green	Blackish greer	Black
5% H_2SO_4	Light green	Dark green	Black
50% HNO_3	Light green	Light green	Dark green

Table: 1. Fluorescent characteristics of *A. solanacea* leaf powder

Phytochemical analysis

Preliminary qualitative phytochemical analysis was performed to confirm the presence of secondary metabolites of different extracts of *A. solanacea*. The results indicated the presence of carbohydrate in hexane, methanol, chloroform, ethyl acetate and dichloromethane extracts. Alkaloid was present in methanol, hexane, acetone, chloroform and dichloromethane extracts. Cardiac glycoside was present in chloroform, methanol, acetone, ethyl acetate and hexane extracts. Saponins were absent in all solvent extracts. Most of the secondary metabolites are present in methanol extract and dichloromethane, acetone and aqueous extracts showed least presence. The presence of phytochemicals present in *A. solanacea* are given in table 2.

Phytochemicals	Solvent extracts ('+' indicate presence and '-' indicate absence)						
	Hexane	Chloroform	Dichloro-Methane	Ethyl-acetate	Acetone	Methanol	Water
Carbohydrate	+	+	+	+	-	+	-
Alkaloids	+	+	+	-	+	+	-
Cardiac Glycoside	-	+	-	+	+	+	+
Coumarin Glycoside	+	-	+	-	+	+	-
Saponins	-	-	-	-	-	-	-
Flavonoids	+	+	-	+	-	+	-
Phytosterols	+	+	+	+	+	+	+
Fats and Oils	-	+	-	-	-	+	+
Phenols and Tannins	+	+	+	+	+	+	+
Proteins	-	+	-	+	-	+	-
Terpenoids	+	+	-	+	-	+	+

Table: 2**.** Phytochemicals present in *A. solanacea* leaf extracts

Percentage extractive

Different plant species would have different phytochemical profile. Chemicals present in the plant materials are dissolved in different solvents for the purpose of further analysis. Therefore, 7 solvents such as hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol, and distilled water were selected to determine the soluble substance. Percentage extractive of hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and aqueous extracts of *A. solanacea* are given in table 3. Methanol extract showed the highest percentage extractive (8.1 %) and hexane, acetone, chloroform extracts showed the lowest percentage extractive value.

Solvents	Hexane	Chloroform	Dichloro methane	Ethyl acetate	Acetone	Methanol	Water
Percentage extractive (%)	1.031	3.53	7.69	6.12	0.047	8.1	5.43

Table: 3. Percentage extractive of leaf extracts

Antibacterial activity

Cold and hot methanol and aqueous extracts were tested against the following bacterial strains: *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa, Vibrio cholerae, Vibrio parahaemolyticus, Xanthomonas campestris* and *P. aeruginosa.* The highest zone of inhibition was observed in methanol hot extract against *S. epidermidis, E. coli, X. campestris* and *K. pneumoniae.* The zone of inhibition for other strains against different solvent extracts are given in table 4. The highest inhibition with an inhibitory zone of 13 mm against *S. epidermidis*.

Bacteria	Diameter of zone of inhibition (mm)			
	Aqueous hot extract	Methanol hot extract	Aqueous cold extract	Methanol cold extract
B. cereus	9	7	Nil	8
B. subtilis	8	7	Nil	7
E. coli	12	Nil	Nil	7
K. pneumoniae	10	8	Nil	9
P. aeruginosa	11	8	Nil	10
S. epidermidis	13	Nil	Nil	7
V. cholerae	10	7	Nil	7
V. parahaemolyticus	8	7	Nil	8
X. campestris	12	7	Nil	7

Table: 4. Antibacterial activity of *A. solanacea* leaf extracts

Antifungal activity

The effects of aqueous and methanolic cold and hot extracts were tested against *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata, Aspergillus niger, Aspergillus tubingensis, Cryptococcus gastritis, Fusarium oxysporum, Phytophthora infestans, Rhizoctonia solani* and *Trichophyton rubrum*. The methanol hot extract showed highest inhibitory zone of 10 mm against *R. solani*. The lowest zone of inhibition is 7 mm showed by methanol hot extract against *T. rubrum*. The results of antifungal activity of *A. solanacea* leaf extracts are given in table 5. Aqueous cold and methanol extract did not show any activity against tested fungal strains. Aqueous hot extracts also show any inhibition against fungal pathogens.

Fungi	Diameter of zone of inhibition (mm)			
	Aqueous cold extract	Methanol cold extract	Aqueous hot extract	Methanol hot extract
A. flavus	Nil	Nil	Nil	Nil
A. fumigatus	Nil	Nil	Nil	8
A. niger	Nil	Nil	Nil	Nil
A. tubingensis	Nil	Nil	Nil	Nil
A. alternata	Nil	Nil	Nil	Nil
C. gastricus	Nil	Nil	Nil	Nil
F. oxysporum	Nil	Nil	Nil	9
P. infestans	Nil	Nil	Nil	Nil
R. solani	Nil	Nil	Nil	10
T. rubrum	Nil	Nil	Nil	7

Table: 5. Antifungal activity of *A. solanacea* leaf extracts

Discussion

Plant extracts are known to have phytochemicals in them possess therapeutic value and can have physiological and pharmacological action on human body. *A. solanacea* is also one such plant which is reported to contain many phytochemicals with therapeutic value. Fluorescence analyses of dried powder of *A. solanacea* were carried out under short, long UV and visible light which signifies their colour characteristics and purity, using successive solvents. The samples of *A. solanacea* showed unique characteristic colours which indicated the absence of any adulteration.

In a similar study by Apraj et al. 2011 in *Citrus aurantifolia*, the leaf powder when treated with different chemicals and the exposed under short, long UV and under visible lights exhibited different colours. The characteristic colours recorded in fluorescent analysis could be used as a standard in the identification and authentication of its crude form and this will further reveal the presence of active agents by their various colour reactions to different chemicals (Apraj et al. 2011). The UV light produces fluorescence in many natural compounds which do not visibly fluorescence in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs. 1958). In addition to this, it will act as a tool to detect adulterants and substituents and will help in maintaining the quality, reproducibility and efficiency of natural drugs (Nair et al. 2005).

The preliminary phytochemical screening of *A. solanacea* revealed the presence of various bioactive compounds such as carbohydrates and secondary metabolites like cardiac glycoside, saponins, flavonoids, phytosterols and phenols in different solvent extracts (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and aqueous). The methanol extract showed the presence of more compounds. Phenols are widely present in all the solvent extracts of *A. solanacea.* It has been reported that phytochemicals present in plants are vital sources of antiviral, antitumor and antimicrobial agents so they are used in allopathic medicine (Nair et al. 2005 and Ramya et al. 2008). Methanolic cold and hot extracts showed significant phenolic and flavonoid contents. Studies conducted by Manach et al. 2005, revealed that phenolic compounds are prominent free radical terminators and are also responsible for physiological functions in animals as well as in plants. The presence of major phytochemicals such as alkaloids, carbohydrate, protein, phenols, flavonoids and tannins in methanol and chloroform extracts of *A. solanacea* were also reported by Islam et al., 2019 which is also in accordance with the present study.

The compounds such as flavonoids which contain hydroxyl groups are responsible for the radical scavenging effect in plants. Anjum et al., 2019 reported the abundance of alkaloids,

carbohydrates, resins, diterpenes, triterpenes, fats and oils in ethyl acetate leaf and stem extract of *A. solanacea* and also reported antioxidant, anti-inflammatory and insect antifeeding activity. Phenols and flavonoids present in *A. solanacea* leaf extract explain its high radical scavenging activity. Knowledge of phytochemical constituents present in *A. solanacea* will be very useful for the maximum exploitation of this plant. In addition to this phenolic compounds are used in several industrial processes for the manufacturing of pesticides, explosives, drugs and dyes. They are also used in the bleaching process of paper manufacturing. Apart from these functions, phenolic compounds have substantial applications in agriculture and forestry as herbicides, insecticides, and fungicides (Santana et al. 2009).

The effect of cold, hot extracts of methanol and water were tested, the aqueous hot extracts showed maximum antibacterial activity with highest zone of inhibition against *S. epidermidis*. Methanolic extracts also showed significant activity. Cold aqueous extract showed no inhibitory zone for all the extracts. Only methanol hot extracts showed antifungal activities against *A. fumigatus*, *F. oxysporum*, *R. solani* and *T. rubrum*. The presence of antimicrobial compound in a particular part of a specific species is due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, phenolic, steroids and saponins etc. (Amin et al., 2015).

The inhibitory action of the leaves against both Gram positive and Gram negative strains indicated the presence of broad spectrum antibiotic compounds in the plant (Siddhuraju and Becker 2003). The proximate composition, nutritional and nutritional factors of *A. solanacea* was reported by Chandran et al. 2015. The presence of Alanine, Glutamic acid and Valine in higher quantities in *A. solanacea* leaf was reported by Chandran et al. 2014. The plant has potential pharmaceutical applications (such as antibacterial and antiviral properties) from extracts of plants from the genus *Ardisia* were noted by Kobayashi and de Mejia 2005. *A. solanacea* leaf extract is also reported to contain antioxidant properties (Chandran et al. 2013). Al-Abd et al., 2017 reported antibacterial activity of methanol leaf extract of *A. elliptica* against *Klebsiella pneumonia* with a zone of inhibition of 9.66 mm. The remaining bacterial pathognes such as *Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, Bacillus cereus, Salmonella typhimurium, E. coli, Pasteurella multocida* did not show any inhibition. In the present study the methanol cold extract and aqueous hot extract

showed antibacterial activity against all the pathogenic bacterial species tested. More specifically, antibacterial activity against four serovars of Salmonella were observed with plant extracts from *A. solanacea* (Phadungkit and Luanratana 2006) and there were positive effects on the reduction of breast cancer cells (Moongkarndi et al. 2004). In addition to the above said properties Islam et al., 2019 reported anxiolytic and sedative, analgesic, hypoglycemic and cytotoxic properties of methanol leaf extracts of *A. solanacea.*

Conclusion

Fluorescence analysis of dried powder of *A. solanacea* showed unique colours which vary pale green to dark green under different solvents. The uniqueness in colour characteristics indicates any adulteration in the sample. Methanol extracts showed the highest percentage extractive value and acetone extracts showed the lowest percentage extractive value. Among the cold, hot aqueous and methanolic extracts tested against nine different strains of bacteria, the aqueous hot extracts showed highest antimicrobial activity against *S. epidermidis.* Aqueous cold extracts showed no activity against the test bacterial strains. Only hot methanolic extract showed inhibition against four fungal strains with highest activity against *R. solani*.

Acknowledgement

The authors sincerely thank Dr. V.V. Pyarelal, Director, K.V.M. College of Science and Technology, Cherthala, Kerala, India, for providing necessary facilities and support for conducting this research work.

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