

**Research Article****Phytochemical and antibacterial analysis of two morpho-types of *Solanum melongena* var. *insanum* (L.) Prain. an ayurvedic herb****R. Thulasi, K. Krishnakumar***

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The Solanaceae, one of the largest family in angiosperms have high commercial value. The genus *Solanum* in Solanaceae is a complex one. Many species in *Solanum* does not contain a well-defined species boundaries. Many of them with taxonomical controversies. The present study was about such a *Solanum* species – *Solanum melongena* var. *insanum*. There were two morpho-types which are botanically known as *S. melongena* var. *insanum*. In Ayurveda both of them were treated as separate plants – as ‘Cheruvazhuthina’ and ‘Punyahachunda’. ‘Cheruvazhuthina’ was extensively used in Ayurveda but ‘Punyahachunda’ was not. Roots of ‘Punyahachunda’ was used instead in the unavailability of roots of ‘Cheruvazhuthina’. So this study was aimed to analyse the pharmacognostic properties of both morpho-types. The therapeutic value was assayed by antibacterial activity of roots of both plants. For convenience the two plants viz., ‘Cheruvazhuthina’ and ‘Punyahachunda’ were denoted as morpho-type 1 and morpho-type 2 respectively. Phytochemical profiling revealed significant differences in certain components. Hence the samples were subjected for FT-IR analyses and the spectra showed high pattern of similarity as well as differences in both the morpho-types investigated. Phytochemical profile was prepared for both the morpho-types which were further fractionated by chromatography. The fractionated components were subjected for bioassay against the growth inhibition of *E. coli*. The bioassay results revealed that the therapeutic potency of morpho-type 1 was more significant and hence I recommend the use of morpho-type 1. However a detailed phytochemical as well as molecular profiling is warranted to confirm the recently obtained results.

Introduction

From the origin of human, man depend on plants for a variety of purposes such as food, medicine, etc. The natural products obtained from various sources including plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates used as medicine throughout the world in traditional system of medical practises [1]. Among these traditional practises most popular practice, the Ayurveda use plants for making various forms of medicines. Secondary metabolites of plants proved to be the principles behind this. They protect plants against infectious diseases, abiotic stress, seed dispersal etc. [2]. *Solanum* sp constitute an ingredient of many Ayurvedic preparations such as ghritham, decoction, arishtam, choornam etc. Different species of *Solanum* are interchangeably used in various preparations. A recent study based on some of the morphological features distinguished the two species of *Solanum* [3]. However their pharmacognostic principles are not investigated. It is important to identify the principles as

well as the genus for identification and usage of plants for medicinal preparations, because specific plant species contain specific pharmacologically important compounds [4].

The present investigation focused on two morpho-types of *Solanum* to identify its uniqueness if any in the Ayurveda and also to assess its pharmacognostic value. There are two morpho-types of *Solanum*, the ‘Cheruvazhuthina’ and ‘Punyahachunda’ (in Malayalam) treated as two different plants in Ayurveda. In Ayurveda system ‘Cheruvazhuthina’ is included in ‘Dasamool’ as an important medicinal plant in the medical treatise. In Sanskrit it is known as ‘Brihati’ and its root is extensively used. But ‘Punyahachunda’ is not used in Ayurvedic preparations. Its fruit is traditionally used in temples for the preparation of ‘punyaham’, the holy water and the female receptacle is used to give red colour to the eyes in Kathakali costumes, a unique traditional art form of Kerala. In certain places roots of ‘Punyahachunda’ is used as a substitute

for roots of ‘Cheruvazhuthina’. But taxonomically these plant specimens are treated as ecotypes of a single plant variety – *Solanum melongena* var. *insanum*(L.)Prain [5]. Both of these plants show many differences in morphology, but considered as same plant variety of a species. Hence the aim of the present investigation is to assess its pharmacological potential. In the present study the two plants are considered as two morpho-types of *S. melongena* var. *insanum*. For convenience ‘Cheruvazhuthina’ is denoted as morpho-type 1 and ‘Punyahachunda’ as morpho-type 2 throughout this work.

Materials and Methods

The samples for the present investigation were collected from different localities of Kerala. These were collected from the localities viz. Nhangattiri, Thirumittacode and Kootanad of Palakkad district, Guruvayur of Thrissur district and Evoor of Alappuzha district of Kerala. The collected samples were identified by the standard procedure followed in taxonomy, based on morphological characters, with the help of Centre for Medicinal Plant Research of Kottakkal Arya Vaidyasala, Kottakkal. And the voucher specimens were prepared and

deposited in the Herbarium of Department of Botany, Sree Neelakanta Govt. Sanskrit College, Pattambi.

Phytochemical assay of two morpho-types

Phytochemical assay of the two morpho-types of *Solanum melongena* var. *insanum* were prepared by the procedure described elsewhere [6]. All reagents used were of AR grade of reputed chemical companies. The extraction solvents were Methanol, Ethanol and Ethyl acetate. The plant materials, both roots and fruits of the two plant types, were shade dried and powdered. 10g powder suspended in 100ml of each of the solvents in a conical flask and kept in a rotary shaker, for thorough mixing, for three days. After that this was filtered out and the solvent along with the dissolved components were allowed to evaporate in Petri plates. After the evaporation of the solvent, the extract was removed from the Petri plates and refrigerated at 4°C for further studies. These extracts were used for different phytochemical analyses [7].

1g of each extract dissolved in 20ml of respective solvents was used for detecting the presence of phytochemical constituents by carrying out the chemical tests described in Table 1.

Table 1: Phytochemical constituents analysed for two morpho-types of *Solanum melongena* var. *insanum*.

Phytochemical constituents	Phytochemical test	Method
Carbohydrates	Molisch’s test	To the aqueous extract add 2 drops of Molisch’s reagent and gently pour along the sides of the test tube Conce. H ₂ SO ₄ and keep the tubes erect for some times.
	Benedict’s test	Take 5ml of Benedict’s reagent and add plant extract to it. Boil for 2 minutes and cool for some time.
	Fehling’s test	Take 2ml each of Fehling’s solution A and B in attest tube, mix them well and boil. Add 1ml of the sample solution drop by drop and boil simultaneously
Protein	Biuret test	Take 3ml of the aqueous extract, add equal volume of Biuret reagent
	Nitric acid test	Take 3ml of Conce. HNO ₃ in a test tube. Add the aqueous extract slowly along the test tube
Alkaloids	Wagner’s test	Dissolve a little of the extract to its solvent and add Wagner’s reagent
	Marquis test	To the sample solution add 3ml of conce. H ₂ SO ₄ and 2 drops of 40% formaldehyde
Steroids	LeibermanBurchard reaction	Add freshly prepared LeibermanBurchard reagent to the extract
	Salkowski test	Extract dissolved in 3ml of chloroform and shaken with 3ml H ₂ SO ₄ .
Flavonoids	NaOH test	To 1ml of sample add 3ml of dilute NaOH, the sample turns yellow. Add dilute HCl, yellow colour disappers
	H ₂ SO ₄ test	To 1ml extract add 1ml Conce. H ₂ SO ₄
Phenol	FeCl ₃ test	Add 3 drops of FeCl ₃ (5% v/v) to 5 drops of sample soluton taken in a test tube
Cardiac glycoside	Keller Killani test	Take 5ml extract in a test tube. Add 2ml of acetic acid along with 1 drop of FeCl ₃ solution and 1ml of Conce. H ₂ SO ₄
Coumarins	FeCl ₃ test	Take 1ml of sample and add 1ml FeCl ₃
	Fluorescence test	1g of extract placed on a slide and covered with filter paper moistened with dilute NaOH, heated on water bath for few minutes

Terpenoids	Salkowski test	Take 1ml sample solution. Add a few drops of chloroform along the sides of the test tube. Add 1 drop of Conce. H ₂ SO ₄
Saponins	Froth test	Take 1ml sample and add 2ml distilled water and shake well
Chalcones	H ₂ SO ₄ test	The sample solution is treated with Conce. H ₂ SO ₄
Tannins	FeCl ₃ test	Add FeCl ₃ (0.1% w/v) to 1ml of sample solution taken in a test tube
Phlobatannins	HCl test	Boil 1ml of extract taken in a test tube with 1% HCl
Acids	Sodium bicarbonate test	A few drops of sample dissolved in 1ml of methanol and slowly added to 1ml saturated solution of sodium bicarbonate.

Thin Layer Chromatography (TLC)

The methanolic extract of root and fruit of each morpho-types were subjected to Thin Layer Chromatography. The different solvent systems of different polarities were prepared and TLC studies were carried out [8].

For separating flavonoids used chloroform: methanol in 5:1 ratio as solvent system.

For separating alkaloids used methanol: ammonium hydroxide in 17:3 ratio as solvent system

For separating saponins used chloroform: glacial acetic acid: methanol: water in 6:2:1:1 ratio as solvent system.

Preparation of TLC plate: 5g of silica gel G was suspended in 10ml of distilled water. Spread it on a microscopic slide for forming thin layer. After 1 hour this TLC plate is transferred to hot air oven and bake it for 2 hours in 120°C.

Loading of sample on TLC plate: 10 µl of the sample (0.1 mg/ml of methanolic extract in methanol) was applied 1cm away from the bottom of the TLC plate by using capillary tubes and developed in a TLC chamber using suitable mobile phases as mentioned above. The developed TLC plates were air dried and observed under UV. The movement of the analyse was expressed by its retention factor (Rf). Values were calculated for different samples.

$R_f = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$

Rf – Retention factor

Fourier Transform Infra-Red spectroscopy (FT-IR)

FT-IR analysis was performed by the procedure described by Diem (1994) [9] and Urban (1994) [10]. The absorbance spectra were measured between 300 and 4500 cm⁻¹.

Anti-bacterial analysis of two morpho-types

Antibacterial analysis was carried out using the ethanolic root extract of both morpho-types to compare the antibacterial activity. Antibacterial activity was analysed against *E. coli*. The extract prepared as in the standard procedure described by Abdulrahman et al. (2014) [5]. 100 mg of dried extract was weighed and suspended in 1ml of dimethyl sulphoxide (DMSO) to make a stock solution of 100 mg/ml dose.

The bacterial strain obtained from stock cultures maintained in the Department of Botany, SNGS College, Pattambi were initially procured from P.S.G. College, Coimbatore.

Preparation of nutrient broth medium: Nutrient broth was prepared by suspending 25 gm of Luria Bertani (LB) broth in

1000 ml of distilled water and boiled to dissolve the medium completely. The medium was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and mixed well before pouring. The medium was cooled to 45-50°C and 3 ml was distributed into each test tube.

Preparation of Muller Hinton agar medium: Muller Hinton Agar medium was prepared by suspending 38 gm of Muller Hinton agar in 1000 ml of distilled water and was boiled to dissolve the medium completely. The medium was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and mixed well before pouring. The medium was cooled to 45-50°C and 20 ml was distributed in each sterile petri plate.

Sterilization: The glass wares, forceps, inoculation loop, petri plates, test tubes and conical flasks were washed well with water and autoclaved. Aseptic condition was maintained in all steps of inoculation and incubation.

Inoculation: The stock cultures of microorganisms used in this study were maintained by agar slants at 4°C. Inoculums were prepared by suspending a loop full of bacterial cultures into 3 ml of nutrient broth and were incubated at 37°C for 2-4 hours. The bacterial suspension was poured into petri plates containing 20 ml Muller Hinton Agar medium. Using the L-shaped glass spreader, bacterial suspensions were spread to get uniform lawn culture.

Anti-bacterial analysis by disc diffusion method: The antibacterial activity of ethanolic extract of roots of both morpho-types were determined by disc diffusion method. 20 ml Muller Hinton agar medium was dispensed into the sterile petri plates in aseptic condition. The medium was allowed to solidify. Then 20 microliter of bacterial inoculum was added to petri plates. The bacterial culture was spread on the Muller Hinton agar medium by a sterilized glass spreader.

Sterile discs were taken and each was impregnated with 150µg, 200µg, 250µg and 300µg plant extracts in DMSO using micropipette and allowed to evaporate in the air and then placed in each petri plates. The plates were incubated at 37°C for 24 hours and zones of growth inhibition were closely monitored. After incubation, the antibacterial activity of plant extracts against the microbes were assessed by measuring diameter of the inhibition zone formed. Antibiotic Cefotaxime was used as positive control for comparing antibacterial activity. DMSO was used as **blank**.

Result

The phytochemical constituents were analysed qualitatively. The Table 2 a. and b. showed the results of phytochemical tests.

Phytochemical profile of two morpho-types

Table 2 a: Phytochemical profile of fruits of the two morpho-types of *Solanum melongena* var. *insanum*

Phytochemical constituents	Fruit						
	Cheruvazhuthina (Morpho-type 1)			Punyahachunda (Morpho-type 2)			
	M E			E E	Ea E	M E	E E
Alkaloids	+	+	+	+	+		
Steroids	+	+	+	+	+		
Flavonoids	+	+	+	+	+		
Phenol	-	-	-	-	-		
Phenolic flavonoids	-	-	-	-	-		
Coumarins	+	-	-	+	-		
Cardiac glycosides	+	+	-	+	+		
Tannins	-	-	-	-	-		
Terpenoids	-	-	-	-	-		
Saponins	+	+	-	+	+		
Phlobatannins	-	-	-	-	-		
Chalcones	+	+	-	+	+		
Acids	+	-	-	+	-		

M E: Metanolic extract; E E: Ethanolic extract; Ea E: Ethyl acetate extract; +: Present; -: Absent

The fruits of both morpho-types contain almost similar phytochemical constituents. Alkaloids, steroids and flavonoids were present in three different extracts of both morpho-types. Coumarins, cardiac glycosides, saponin and chalcones were

present in methanolic and ethanolic extract of both morpho-types. Acids present in methanolic extract of both. Phenol, phenolic-flavonoids and phlobatanins gave negative results in both morpho-types. Terpenoids were present in ethyl acetate extract of morpho-type 2 and which was absent in morpho-type 1.

Table 2b: Phytochemical profile of root of the two morpho-types of *Solanum melongena* var. *insanum*

Phytochemical constituents	Root				
	Cheruvazhuthina (Morpho-type 1)			Punyahachunda (Morpho-type 2)	
	M E	E E	Ea E	M E	E E
Alkaloids	+	+	+	+	+
Steroids	+	+	+	+	+
Flavonoids	+	+	-	+	+
Phenol	+	+	-	+	+
Phenolic flavonoids	+	-	-	+	-
Coumarins	+	+	-	+	+
Cardiac glycosides	+	+	-	+	+
Tannins	-	-	-	-	-
Terpenoids	-	-	-	-	-
Saponins	+	+	-	+	+
Phlobatannins	-	-	-	-	-
Chalcones	+	+	-	+	+
Acids	-	-	-	-	-

M E: Metanolic extract; E E: Ethanolic extract; Ea E: Ethyl acetate extract; +: Present; -: Absent

The roots of both morpho-types gave similar results for the phytochemicals investigated. Alkaloids, steroids and flavonoids were present in three different extracts of both morpho-types. Phenol, phenolic flavonoids, coumarins, cardiac glycosides, saponins and chalcones were present in methanolic as well as ethanolic extract of both morpho-types. Tanins, terpenoids and acids were absent in both morpho-types.

Thin Layer Chromatography (TLC)

After the development of TLC, the TLC plate was viewed under UV light of short wave.

TLC plate for the methanolic extract of fruit of both morpho-types developed in the solvent system for flavonoids gave identical bands with Rf values 0.916, 0.75, 0.5 and 0.166.

The flavonoid profile of the methanolic extract of root of both morpho-types gave three identical bands with Rf values 0.916, 0.33 and 0.166. The band with Rf value 0.75 was absent in the root of morpho-type 2 and it possessed a band with Rf value 0.416. The methanolic extract of root of morpho-type 1 contain two bands which were absent in morpho-type 2, with Rf value 0.75 and 0.5.

TLC plate developed in solvent system for separating alkaloids in the methanolic extract of fruit of morpho-type 1

gave bands in Rf values 0.77, 0.67 and 0.2. And that of morpho-type 2 gave bands in Rf values 0.7, 0.5 and 0.14.

The alkaloid profile of methanolic extract of root of morpho-type 1 gave bands in Rf values 0.64, 0.42 and 0.21. And that of morpho-type 2 gave bands in Rf values 0.57, 0.5, 0.42 and 0.14.

The solvent system used for separating saponins gave the following results; the fruit of morpho-type 1 and morpho-type 2 gave bands with Rf values 0.64 and 0.14. Root of morpho-type 1 gave band with Rf value 0.5, and that of morpho-type 2 gave band with Rf value 0.42. Root of both plants give band with Rf value 0.57.

Fourier Transform Infra-Red spectroscopy (FT-IR)

The FTIR analysis showed some peculiar peaks in both the plant specimens. These peaks are unique for each specimen.

Root of morpho-type 1 had peak at 3406 which indicates the presence of -OH bond of alcohol and phenol. This peak is absent in morpho-type 2. The root of Morpho-type 2 had a peak at 2129 cm⁻¹, indicates the presence of C-N containing glycosides.

Fruit of morpho-type 1 had peaks at 1512 cm⁻¹ and 1591 cm⁻¹ of secondary amine alkaloid and 1728 cm⁻¹ of ketones. Morpho-type 2 fruit gave peak at 432 cm⁻¹ of metal-oxygen bond (Fig. 1 a, b, c and d.)

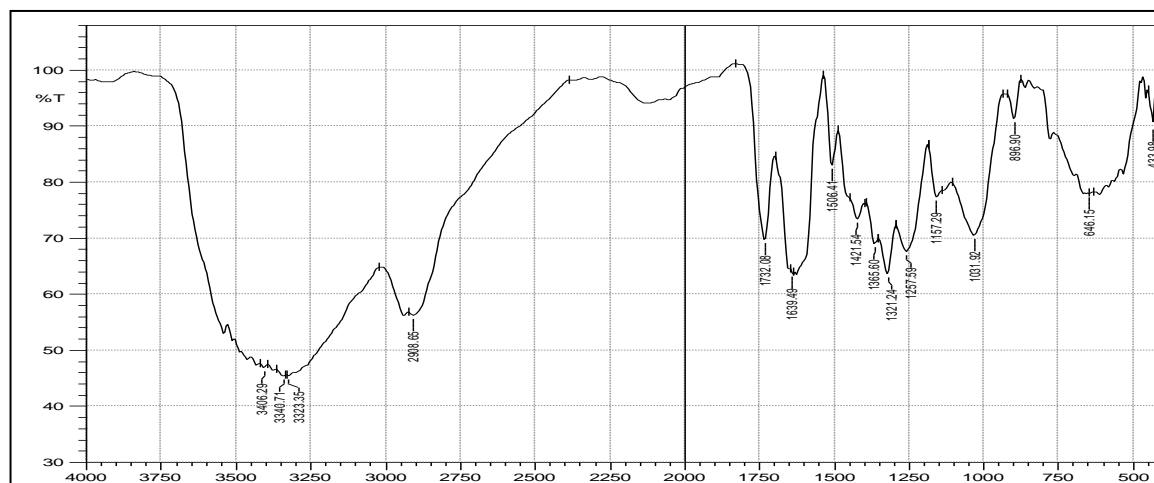


Fig. 1 a. The FT-IR absorption spectra of root of morpho-type 1

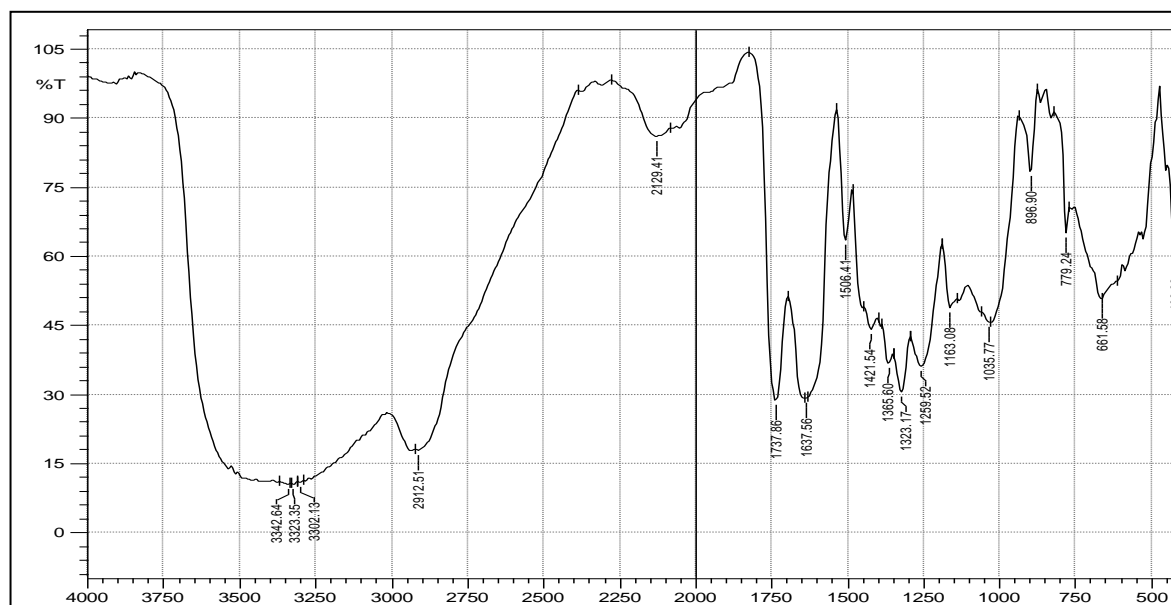


Fig. 1 b. The FT-IR absorption spectra of root of morpho-type 2

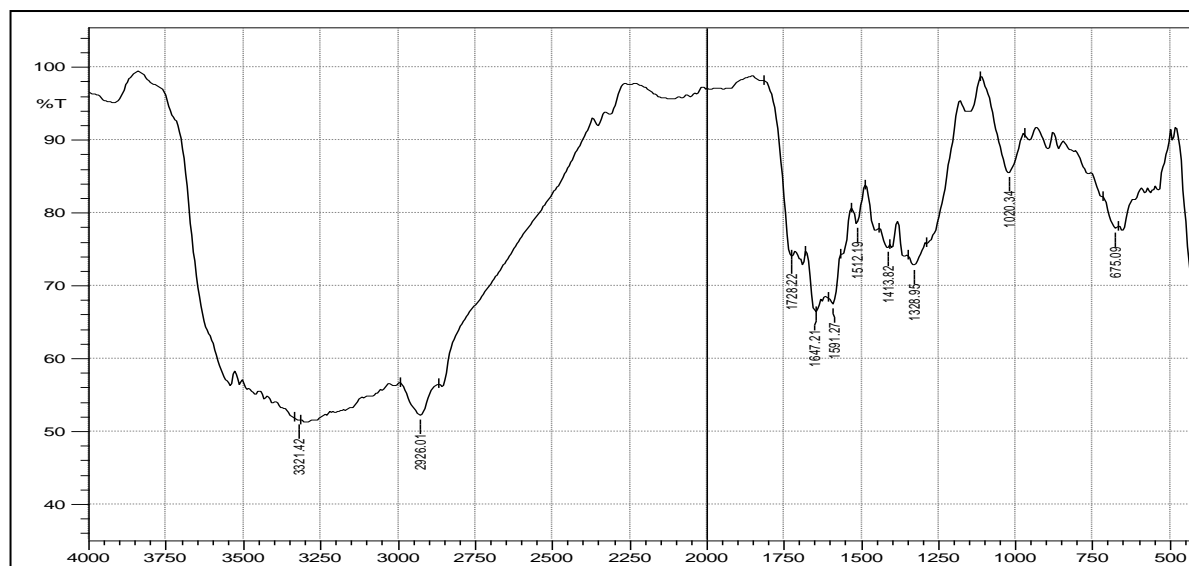


Fig. 1 c. The FT-IR absorption spectra of fruit of morpho-type 1

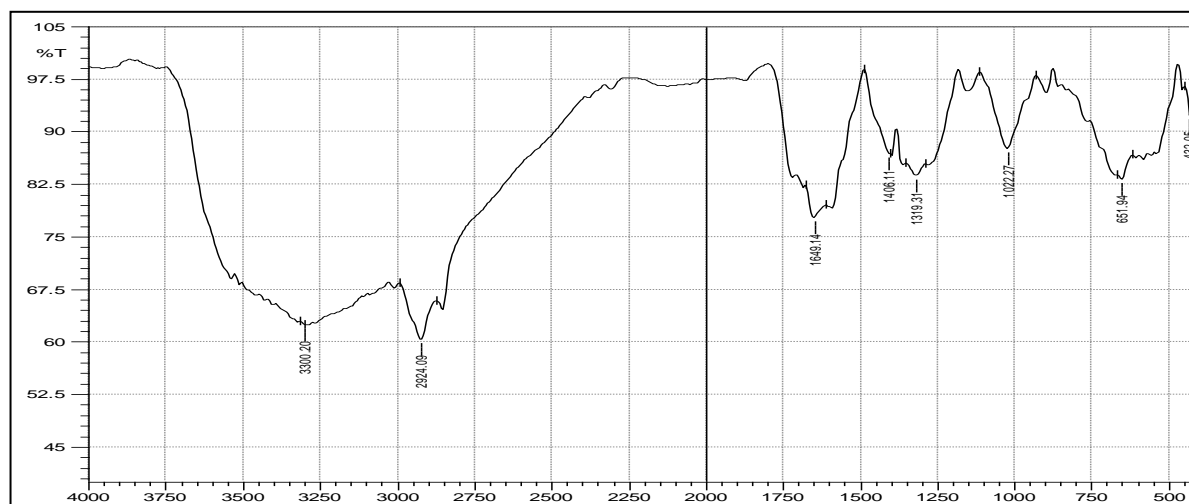


Fig. 1 d. The FT-IR absorption spectra of fruit of morpho-type 2

Anti-bacterial analysis of two morpho-types

The ethanolic root extract of both morpho-types showed antibacterial activity against the bacteria *E. coli*. The measurements of zone of inhibition for the two morpho-types against the bacteria *E. coli* in different concentrations showed in Table. 3.

Table 3. Zone of inhibition of ethanolic extract of two morpho-types of solanum against *E. coli*.

Concentration	Measurement of zone of inhibition	
	Morpho-type 1	Morpho-type 2
150 µg	0.73 cm	0.61 cm
200 µg	0.94 cm	0.75 cm
250 µg	1.22 cm	0.97 cm
300 µg	1.47 cm	1.12 cm

The results showed that the morpho-type 1 had more antibacterial activity than the morpho-type 2.

Discussion

Preliminary phytochemical profiling of two morpho-types was showed no significant differences. Only the difference noticed was the presence of terpenoid in the ethyl acetate fraction of the fruit of morpho-type 2 (Table. 2 a). Both the morpho-types contain almost similar phytochemical profile. On further fractionation by Thin Layer Chromatography revealed significantly different profile as well as certain uniqueness. For TLC methanolic extract of root and fruit of both morpho-types were used. TLC for separating flavonoids showed many similar bands, but one band with Rf value 0.75 is absent in the methanolic extract of root of morpho-type 2. TLC for separating alkaloids gave almost similar bands, except in case

of an additional band with Rf value of 0.5 present in the root of morpho-type 2. TLC for saponins give almost similar bands in both morpho-types. Mostapha and Hayette (2015) [11] reported that phytochemical profiling is used to distinguish the similarity and differences as well as therapeutic potential in plant accessions of *Ficus*. There was a report which discussed about the use of phytochemical constituents, especially flavonoids, to resolve the *Solanum nigrum* species complex [12]. Here in this study, the two morpho-types shows some differences in its phytochemical constituents. This differences may be due to ecological as well as climatic factors. So a further detailed phytochemical investigations are required for confirming the relationship of the two morpho-types investigated. For that we did Fourier Transform Infrared (FT-IR) spectroscopy of both morpho-types.

Fourier Transform Infrared (FTIR) spectroscopy was used to predict the structure as well as chemical bonds present in the given sample [13]. There were many reports in which the FTIR spectroscopy is used for classification of plants at the level of species and varieties [14, 15]. FTIR spectroscopy is a rapid, non-invasive, high resolution analytical tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "finger print" [16]. Tao et al. (2011) [15] used FTIR spectroscopy and chemo metric analysis for classification of five kinds of Moss plants. Yiling (2012) [14] used FTIR for identification of Chinese herb *Solanum lyratum* and classifying it into three ecotypes based on the FTIR results. In the present study FTIR spectroscopy was used for identifying and segregating the two morpho-types of *S. melongena* var. *insanum*. The results revealed that there were some unique peaks for each morpho-type. Root of morpho-type 1 showed peak at 3406 cm⁻¹, indicated the presence of alcohol and phenol bond containing unique phytochemicals in the root of morpho-type 1. The root of morpho-type 2 showed peaks at 2129 cm⁻¹ indicated C-N containing glycosides. Fruit of

morpho-type 2 contain the peak at 432 cm⁻¹ of metal-oxygen bond and that of morpho-type 1 give peak at 1512 and 1591 cm⁻¹ of secondary amine. Rei et al. (2013) [17] distinguished different varieties of wheat using FTIR spectroscopy. The FTIR results depicted that the two morpho- types might be the different varieties in *Solanum melongena*.

In Ayurveda both the collected accessions were alternatively used for various medical preparations as well as for preparing 'punyaha' in temples. The phytochemical profiling revealed that both the morpho-types could be used for ayurvedic preparations. So for testing the effectiveness of the substitution the antibacterial activity of the ethanolic root extract were analysed. The ethanolic root extract was assayed because in Ayurveda this plant is mainly used to make 'Arishtam', an ethanolic preparation. So by analysing the effect of ethanolic root extract against bacterial strains reveals the pharmacognostic potential of the two morpho-types. Most of the antibacterial studies were carried out by using the ethanolic or aqueous extracts, because these are the main solvent systems used in ayurvedic preparations [18]. The root is used in medicinal preparations, so antibacterial analysis was carried out using the root extract. The antibacterial activity of both morpho-types were analysed against the bacteria *E. coli*. (Fig.3) This bacterial strain was selected because of its presence in water bodies and its presence is a parameter for potability of water. The results revealed that the root extract of morpho-type 1 had significant antibacterial activity than the root of the morpho-type 2 (Fig. 3). Edible grains contains principles that could control bacteria like *E.coli*[19]. Hence the use of morpho-type 2 instead of morpho-type 1 might not give better results especially in controlling the *E. coli* during the preparation of 'punyaha' using pond water. However further investigations with more pathogenic strains are warranted to confirm these results.

Conclusion

Phytochemically the two morpho-types contain almost same constituents and certain unique compounds as with the result of FT-IR analysis. There were some peaks unique for each morpho-type. It also pointed to the distinctiveness of the two morpho-types. The morpho-type 1 has more antibacterial activity as compared to morpho-type 2. So in Ayurveda the use of morpho-type 2 instead of morpho-type 1 would not impart expected relief. The two plants can be used to control the water borne pathogenic bacteria *E. coli* in drinking water as a chemical free water purification method.

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