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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLANUM SPECIES AGAINST *HELICOBACTER PYLORI*

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ABSTRACT

Helicobacter pylori is considered as a major causative agent for several gastrointestinal diseases such as gastric and peptic ulcer. The present research deals with the phytochemical analysis and antibacterial effect of different Solanum sps. including Solanum torvum, Solanum xanthocarpum, Solanum nigrum and Solanum trilobatum against Helicobacter pylori. The phytochmeical analysis revealed the abundant presence of the flavonoids, alkaloids and steroids in the leaf extract of Solanum xanthocarpum, Solanum nigrum and Solanum trilobatum. Among the aforementioned Solanum sps., the aqueous extract of Solanum xanthocarpum exhibited the maximum activity followed by the ethanol extract of Solanum torvum and aqueous extract of Solanum nigrum. In addition, the TLC and HPLC analysis also confirmed the presence of flavonoids, alkaloids and steroids in the leaf extract of Solanum xanthocarpum. The presence of phytochemicals like flavonoids, alkaloids and steroids might be responsible for the antibacterial activity against the Helicobacter pylori. The results of the study exemplify the Solanum xanthocarpum as a fine natural herbal medicine for several gastrointestinal diseases.

KEY WORDS

Helicobacter pylori; Solanum xanthocarpum; antibacterial activity; TLC; HPLC.

INTRODUCTION

Human intestinal tract is a host to vast ecology of microbes and harbors more than 500 identified species (Harish and Vargaeesh, 2006). A spiral shaped bacterium which adapt a unique way to survive the harsh environment of stomach and on the lining of intestine indicate the *Helicobacter pylori* (Bizzozero, 1883; Warren J R 1983). *H. pylori* is responsible for causing chronic infection and related diseases in billions of people around the world. It can survive gastric acids due to its ability to produce a urease enzyme. Through a chemical process, urease can neutralize stomach acid, making it easy for the bacteria to survive in its own acid free zone (Thieblemont, 2000).

About half of the world's population in the developing countries are infected with a very high prevalence of gastric ulcer and impose a key burden to the health care systems worldwide. The most common regimen combination used for the treatment of *H. pylori* infection is of proton pump inhibitors (PPI), amoxicillin and clarithromycin (triple therapy). The control *H. pylori* infection and its treatment has become more complicated because of the high emergence of high resistance to the drugs used (Backert *et al*., 2004; Peek 2005). Due to the negative impact of synthetic medicine (chemicals), many natural plants with anti-*H.pylori* activity have been explored to eradicate the gastrointestinal infections (Ndip et al., 2008; Vorvathikuchai and Mitchell, 2008).

The Solanum sp. such as *Solanum xanthocarpum*, *Solanum nigrum*, *Solanum trilobatum* and *Solanum torvum* are widely used traditional plants in oriental Indian medicine (Jain et al., 1968; Null 2001). The anti-oxidant, antitumorogenic, antiinflammatory and antipyretic activity of this Solanum plant makes it more influence in the field of traditional medicine (Jain et al., 1968; Jainu M et al 2006). In this present study, the

antibacterial activity of different leaf extracts of *Solanum xanthocarpum*, *Solanum nigrum, Solanum trilobatum* and *Solanum torvum* was examined against *H. pylori*.

MATERIALS AND METHODS

Collection of Plants and extract preparation

The four-different species of Solanum plants were collected for the present study from the nearby areas of Tirunelveli district. The preparation of plant extracts were carried out based on the method of Razmavar et al., 2014. The plant parts especially leaves were dried at 56ºC in an oven until the full moisture content got reduced. The leaves were grounded to fine powder and all the extraction was carried out at room temperature. The powders were soaked in sterile distilled water followed by absolute ethanol and petroleum ether in ratio of about 1:20. After 10 days, the extracts were filtered through Whatmann filter paper and evaporated using vacuum to concentrate the extract. The collected extracts were stored at 4ºC for further use.

Phytochemical Analysis

1. Steroid Test

About 2ml of acetic anhydride was added to 0.5g of plant extract to which 2ml of sulphuric acid was added along the sides of the test tubes. Observation of color change from violet or blue green indicated the positive result.

2. Glycoside Test

About 1ml of glacial acetic acid containing traces of ferric chloride and one ml of concentrated sulphuric acid was added to 0.5 ml of plant extracts (alcoholic extracts). Formation of reddish brown color at the junction and its change to bluish green in the upper layer indicated the presence of glycosides.

3. Flavonoids Test

About 5-10 drops of diluted HCl was added to a test tube containing 0.5 ml of plant extract. To this a small piece of $ZnCl_2$ or magnesium was added and boiled for few minutes. Appearance of reddish brown color indicated the presence of flavonoids.

4. Tannin Test

About 50 mg of respective plant extract was dissolved in 20 ml of distilled water and boiled in a test tube and filtered. To this filtrate, few drops of 0.1% of ferric chloride solution was added. Formation of a blackish blue colour indicated the presence of tannin.

5. Saponin Test

About 50 mg of plant extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. Development of a two cm layer of foam indicated the presence of saponins.

6. Phytosterols Test

Small portion of the plant extract was dissolved in chloroform. To this few drops of acetic anhydride along with few drops of concentrated sulphuric acid was added along the sides of the tubes. Formation of blue to blood red color indicated the positive result.

7. Phenolics Test

About 50 mg of plant extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. Appearance of a dark green colour indicated the presence of phenolic compounds.

8. Alkaloids Test

About 50 mg of solvent free plant extract was stirred with a few ml of diluted HCl and filtered. To 1.2 ml of filtrate, 0.1 ml of Mayer's reagent was added. Occurrence of a white creamy precipitate indicated the presence of alkaloids.

9. Terpenoids Test

About 0.5 g of the plant extract was added to a 2ml of chloroform. To this 3ml of the concentrated sulphuric acid was carefully added to form a layer. Formation of a reddish brown colour in the interface indicated the presence of terpenoids.

Antibacterial Activity

Well Diffusion Method

The test organism *H. pylori* was purchased from American Type Culture Collection, USA (ATCC 26695). A 100µl of the test organism was spreaded uniformly on Muller Hinton blood Agar plates. After inoculation, wells were prepared using sterile cork borer. About, 50µl of the leaf extracts of different plant solvents were transferred into each well. Solvents without plant leaf served as a control. The plates were then allowed to stabilize for about 1 hour and incubated at 37°C for 2-3days in microaerobic condition. The experiment was carried out in triplicates.

Separation and identification of Plant compounds

Separation of active compounds by TLC

The active compounds separation from the crude plant extracts were carried out with silica gel. The extract was spotted on the silica gel plates and allowed to test against the following solvent system hexane, diethyl

ether, petroleum ether, ethyl acetate, chloroform and water was used with varied combinations of solvents according to the polar basis. The developed plates were observed under UV light to visualize fluorescent absorbing bands.

RESULTS AND DISCUSSION

Phytochemical Study

The basic phytochemical studies were carried out to check the presence of plant components in the selected plants of Solanum species. The leaves were used to examine the phytochemical properties of the plants. Nine different phytochemical analysis were carried in this study (Table 1).

Table: 1 Phytochemical analysis of the leaf extracts of Solanum plants

Plants	Solvents	Phytochemical Tests								
		Ster.	Flav.	Alk.	Phe.	Glyc.	Sap.	Tan.	Terp.	Phyt.
Solanum torvum	Pet. ether	+	+	+	+	-	-	-	+	-
	Chloroform	+	+	+	+	+	+	-	+	-
	Acetone	+	+	+	+	+	-	-	+	+
	El. acetate	-	-	+	-	+	+	-	-	-
	Methanol	+	+	+	+	-	+	-	+	+
	water	+	+	+	+	-	+	-	-	+
Solanum xanthocarpum	Pet. ether	+	+	+	-	+	-	-	-	+
	Chloroform	+	+	+	+	+	+	+	+	+
	Acetone	+	+	+	+	-	-	-	+	-
	El. acetate	-	+	+	-	-	+	+	-	-
	Methanol	+	+	-	+	+	-	-	+	+
	water	+	+	-	-	+	+	-	+	+
Solanum nigrum	Pet. ether	+	+	+	-	-	-	+	-	-
	Chloroform	+	-	+	-	+	-	+	-	-
	Acetone	+	+	+	+	+	+	+	+	+
	El. acetate	-	+	+	+	+	+	-	-	-
	Methanol	+	+	+	-	+	-		-	+
	water	+	+	+	+	+	+	+	+	+
Solanum trilobatum	Pet. ether	+	+	-	-	-	+	+	-	+
	Chloroform	+	+	+	+	+	+	-	-	+
	Acetone	+	+	-	+	+	-	+	-	-
	El. acetate	-	+	+	+	-	+	-	+	+
	Methanol	+	+	+	+	+	+	-	+	+
	water	+	+	+	-	+	+	+	-	+

Ster. – Steroid; Flav.- Flavnoid; Alk.- Alkaloid; Phe. – Phenolic; Glyc.-Glycoside; Sap.- Saponin; Tan.- Tanin; Terp.- Terpenoid; Phyt. – Phytosterol. '+' Positive; '-' Negative.

Table: 2 Antibacterial activity of Solanum plant extracts against *H. pylori*

Name of the Plants	Zone of inhibition of plant extracts mm*					
	Petroleum ether	Chloroform	Acetone	Ethyl acetate	Methanol	water
Leaf extract						
Solanum torvum	-	10.4±0.2	-	-	9.2±0.1	-
Solanum xanthocarpum	-	10.2±0.1	12.2±0.1	-	13.2±0.1	-
Solanum nigrum	-	8.1±0.1	6.2±0.1	6.0±0.1	11.2±0.1	-
Solanum trilobatum	-	8.3±0.2	-	-	8.2±0.1	-

Figure 1 - Plants A. *Solanum trilobatum* B. *Solanum. torvum* C. *Solanum xanthocarpum* D. *Solanum nigrum*

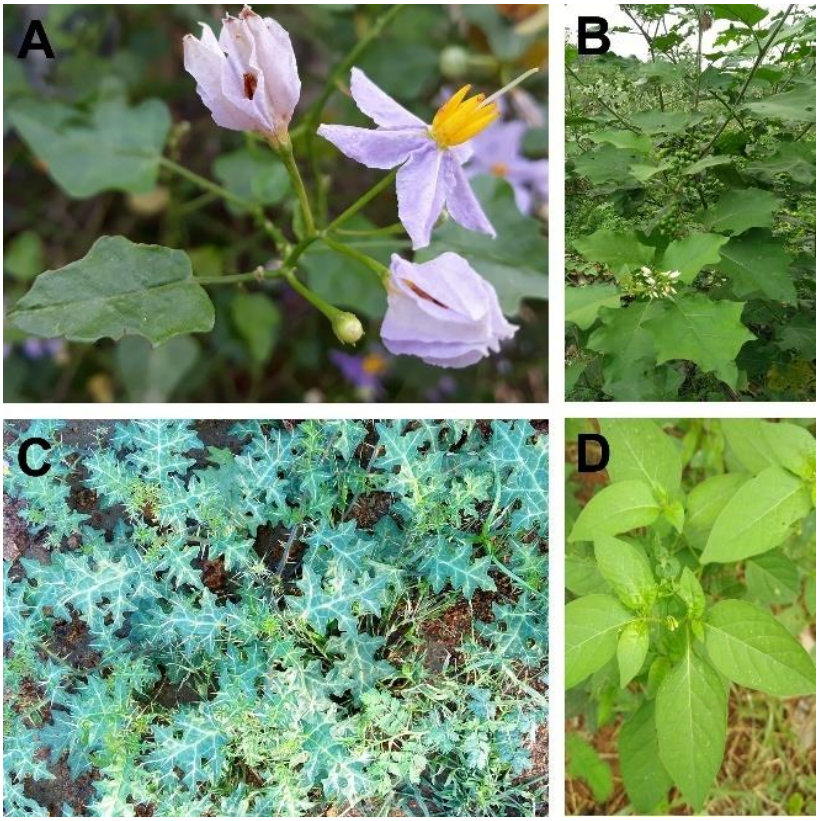
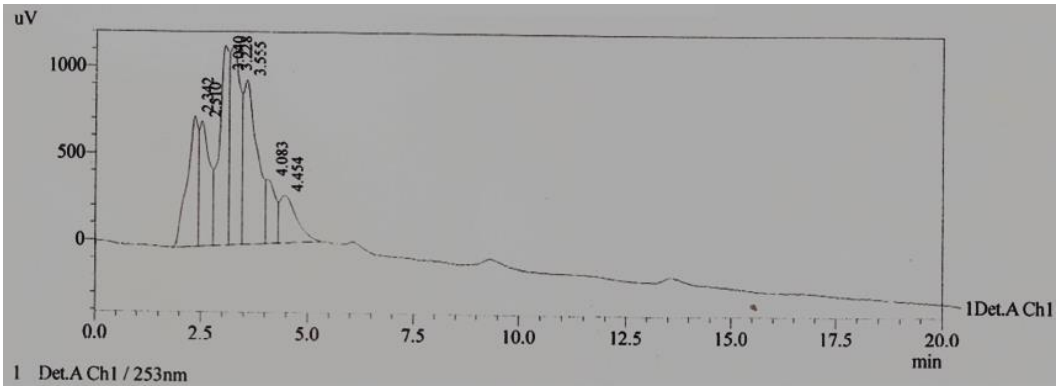


Figure 2 - Thin Layer Chromatorgraphy of Methanol extract of *Solanum xanthocarpum*

Figure 3 - HPLC analysis of partially purified *Solanum xanthocarpum* plant extract



In well diffusion method, different solvents of the four selected Solanum plant extracts were treated against *H. pylori*. The methanol extract of *Solanum xanthocarpum* exhibited a maximum zone of inhibition against *H. pylori*. Followed by *Solanum xanthocarpum*, the *Solanum nigrum*, *Solanum trilobatum* and *Solanum torvum* represented a moderate zone of inhibition against *H. pylori*. Likewise, methanol and chloroform extracts from plant leaves rendered fine antibacterial activity against *H. pylori*. Herein, *Solanum xanthocarpum* and *Solanum torvum* obtained a maximum zone of inhibition compared to *Solanum nigrum* and *Solanum trilobatum* against the *H. pylori*. *Solanum xanthocarpum* and *S. nigrum* showed the inhibition zones in acetone extract. The *H. pylori* rendered resistant ability against the acetone extract of *Solanum torvum* and *Solanum trilobatum*. The ethyl acetate extract of *Solanum nigrum* inhibited *H. pylori* whereas petroleum ether and water extract did not develop any inhibitory zones against *H. pylori* clearly indicating the potent resistant capability of *H. pylori* against the test extracts. The selection of solvents and plant species is of most potential steps in the phytochemical studies (Kusumoli et al., 1995). In the present study, the methanol extract showed maximum activity against *H. pylori.* The results corroborated with the findings of Franklin et al., 2012 suggesting the high susceptibility of *H. pylori* to the ethanolic extracts of *Emblica officinalis.* The anti-*H.pylori* compound eluted from the ethanolic extract of *Terminalia chebula* showed higher activity when compared to the other polar sovents (Sato et al., 1997).

The highest zone of inhibition was observed at polar solvent especially methanol and acetone which may be due to the dipole movement of the polar solvents in the *Solanum xanthocarpum*. The phytochemical like flavonoids present in the leaf extract may tend to bind with the cell wall membrane of the *H. pylori* leading to the disruption of the cell membrane. Consequently, it resulted in the improper membrane transport ultimately ending in cell death.

The active components of *Solanum xanthocarpum* plant extract was separated by using thin layer chromatography method. Herein, the mobile phase was used with the combination of various solvents such as cholorform:ethanol to separate the active compounds (C1, C2, C3).

The HPLC analysis of leaf extract of *Solanum xanthocarpum* showed peaks at different retention time (mins) such as 2.34, 2.51, 3.04, 3.22, 3.55, 4.08 and 4.45 mins corresponding to the presence of flavonoids, phenols, tannins, casdiac glycosides, saponins and coumarin respectively. Among the peaks, peak at the retention time 3.040 and 3.228 mins rendered a maximum peak area compared to the other peaks. The high area peaks indicated the presence of flavonoids and alkaloids in the leaf extract of *Solanum xanthocarpum*.

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