WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 8, Issue 1, 732-740 Research Article ISSN 2278 – 4357

INVITRO PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *SOLANUM MURICATUM* IN HYDROALCOHOL EXTRACT

Mohamed Halick A.[1*], Sanjay Kumar T.[1], Yuvaraj K.[1], Ramesh Babu N. G.[2] and Dr. Saravanan N.[3]

[1,2,3]Department of Biotechnology, Adhiyamaan College of Engineering (Autonomous), Hosur – 635109, Tamil Nadu, India.

Article Received on 25 October 2018,

Revised on 15 Nov. 2018,
Accepted on 06 Dec. 2018,

DOI: 10.20959/wjpps20191-12630

***Corresponding Author**

Mohamed Halick A.

Department of Biotechnology, Adhiyamaan College of Engineering (Autonomous), Hosur – 635109, Tamil Nadu, India.

ABSTRACT

The Phyto nutrients present in the leaves of *Solanum muricatum* herb was extracted using hydro alcohol as a solvent. Preliminary phytochemical analysis was carried out for extracts and leaf extracts reveal the presence of alkaloids, flavonoids, saponin, phenols, terepnoids, tannins, carbohydrates. The susceptibilities of these test bacteria on the extracts was determined using the Minimum Inhibitory Concentration (MIC), Zone of Inhibition Test (ZIT) methods. These antioxidant properties was usefully exploited for medicinal purposes.

KEYWORDS: *Solanummuricatum*, Antioxidant,MIC, ZIT, Phytochemical screening.

INTRODUCTION

Solanum muricatum is a species of evergreen shrub which belongs to the family of Solanaceae in the order of Solanales. It is a sweet edible fruit. It is known as pepinodulce or simply pepino; the latter is also used for similar species such as S. mucronatum. The pepinodulce fruit resembles a melon in color, and its flavor recalls a succulent mixture of honeydew and cucumber, and thus it is also sometimes called pepino melon or melon pear, but pepinos are only very distantly related to melons and pears. (Yang Wang, Yanping Wu).

Origin

Solanum muricatum is a evergreen shrub native to South America.

Distribution

The pepinodulce is presumed to be native to the temperate Andean regions of Colombia, Peru and Chile though it is not known in the wild and the details of its domestication are unknown. The pepino is a domesticated native of the Andes. Pepino plants are cultivated in temperate regions of New Zealand, California.

Description

It can grow upto 3-foot or so shrub and the foliage looks very similar to that of potato plant while it's growth habit is akin to that of tomato and for this reason often, require staking. The plants will flower from August to October and fruits appear from September through November. Fruit from the growing Pepino plants may be round, oval or even pear shaped and may be white, purple, green or ivory in colour with purple striping.

The eudicotyledons are a clad of flowering plants that had been called tricolpates or non-magnoliid dicots. The flowers of pepino plants are hermaphrodites, having both male and female organs, and are pollinated by insects. Cross pollination likely results in hybrids and explaining the vast differences between fruit and foliage among growing pepino plants. The plants may be grow in sandy, loamy or even heavy clay soils, although they prefer alkaline, well-draining soil with an acid neutral pH. It should be planted in sun exposure and moist soil.

Pepino plant do not set fruit until the night temperatures are over 18C(65 F). The fruit matures 30-80 days after pollination.

In previous works they have shown basic studies on the selected species.In our study the antibacterial and phytochemical analysis of *Solanum muricatum* was carried out as preliminary test. The antioxidiant by using DPPH and Radio Scan assay.

MATERIALS AND METHODS

Sample collection and preparation

The *S. muricatum* leaves were colleted freshly from Yerkad forest near Salem and was shade dried for 5 days. The Sun dry method was avoided, since it may decrease the ingredients present in the leaves. 10gms of well dried and powdered leaves was taken and boiled with 100ml methanol (100%). The crude solution was filtered and used as extract for the analysis.

Reagent Preparation

Working Stock solutions are prepared by diluting the stock solutions with double distilled water. Nutrient agar for bacteriological culture was obtained from Hi-media, Mumbai.

Analysis of *Solanum muricatum*

Phytochemical Screening

Preliminary phyto chemical analysis was carried out for the methanol extract of *solanum muricatum* leaves as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.
b) **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.
b) **H_2SO_4 test**: Extracts were treated with few drops of H_2SO_4. Formation of orange color indicates the presence of flavonoids.

Detection of Steroids

Liebermann- Burchard test: 2ml of acetic anhydride was added to 0.5g of the extract, each with 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Detection of Phenols

a) Ferric chloride test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Pottassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Antioxidant Activity

Reducing Power Assay

The sample together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20min (Liyana-Pathirana CM). The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloro acetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance was detected at 700nm after reaction for 10min. The higher the absorbance represents the stronger the reducing power. The reducing power assay was expressed in terms of Ascorbic acid equivalent per gram of dry weight basis as per (Ramadan-Hassanien MF (2008).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation:

$$IC = \frac{(Absorbance\ of\ control - Absorbance\ of\ test)}{Absorbance\ of\ test} * 100. IC50$$

RESULTS AND DISCUSSION

A. Phytochemical Analysis of *solanum muricatum*

Table 1: Phytochemical Analysis and Results.

Phytochemicals	Observations	Extracts
		Methanol
Alkaloids		
Mayer's test	Cream color	+
Wagner's test	Reddish brown solution/ precipitate	+
Flavonoids		
Lead acetate test	Yellow orange	+
H_2SO_4 test	Reddish brown / Orange color precipitate	+
Steroids		
Liebermann-Burchard test	Violet to blue or Green color formation	-
Terpenoids		
Salkowski test	Reddish brown precipitate	+

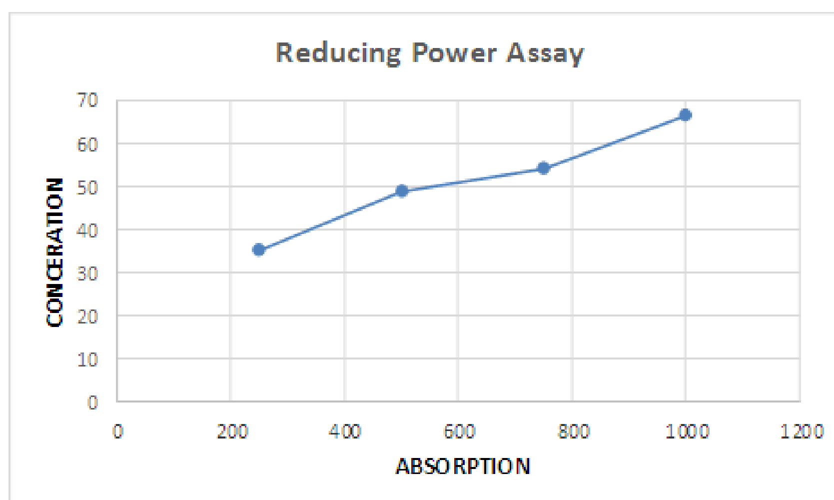
Arthroquinone Borntrager's test	Pink color	-
Phenols Ferric chloride test Lead acetate test	 Deep blue to Black color formation White precipitate	 + +
Saponin	Stable persistant	+
Tannin	Brownish green / Blue black	+
Carbohydrates	Yellow / brownish / blue / green color	+
Oils & Resins	Filter paper method	-

Fig. 1: *Solanum muricatum* phytochemical analysis.

Graphs: Concentration (in µg/ml) (x – axis) Vs MIC Diameter (in mm)(Y – axis)

Table 2: Reducing power assay of *Solanum muricatum*.

S. No.	Concentration	OD	% IC_{50}	IC_{50}
1		0.209	42.17	615.82
2	500	0.218	48.29	
3	750	0.225	53.06	
4	1000	0.231	57.14	

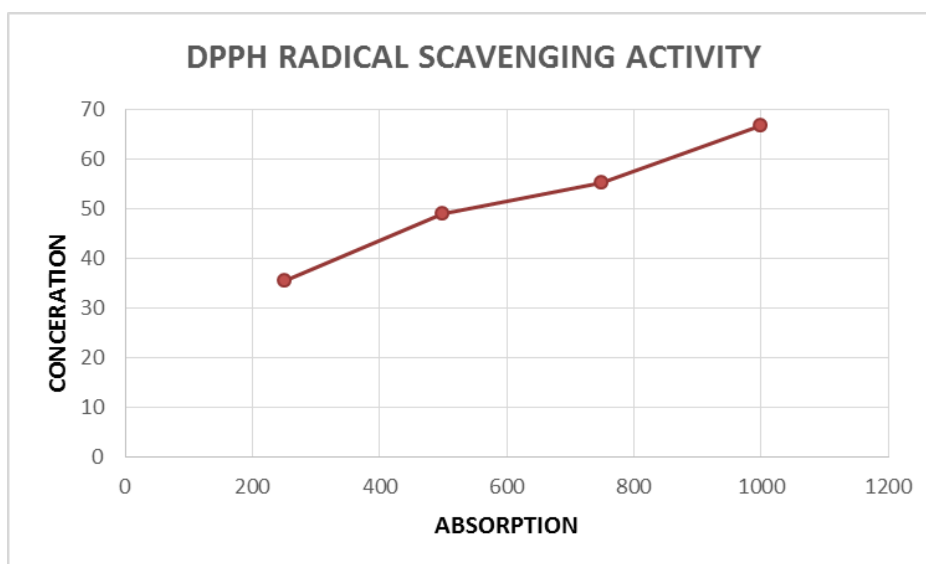


Graph 1: Reducing power assay.

The graph shows that the extracts of selected plant species possess reducing properties` the peeks are due to the ability of plant extract to act as a reducing agent. The graph plotted is concentration of extract (y – axis) against the absorption (x – axis) O.D.

Table 3: DPPH assay of *Solanum muricatum.*

S. No.	Conc	OD	% IC_{50}	IC_{50}
1	250	0.199	35.37	586.87
2	500	0.219	48.97	
3	750	0.228	55.10	
4	1000	0.245	66.66	



Graph 2: DPPH assay.

CONCLUSION

Phytochemical screening of *Solanum muricatum*that it has sufficient phytochemicals needed for the medicinal properties for drug productions. Methanol extract of the leaves of the *Solanum muricatum*was subjected to phytochemical analysis and it reveals the presence of phytochemicals which are rich in medicinal properties and it can be used for drug productions. (Rajput *et al* (2011)) The presence of phytochemicals was confirmed by phytochemical analysis. Ampicillin was used as control for disc DPPH and reducing power assay method At 250, 500, 750, 1000(mg/ml) concentration.

This study estimated that 1000 (mg/ml) is the suitable concentration for inhibiting the bacterial species. At 1000 (mg/ml) radial scavenginghas been estimated with a %IC of about 57.14,At 750 (mg/ml) radial scavenginghas been estimated with a %IC of about 53.06,At 500 (mg/ml) radial scavenginghas been estimated with a %IC of about 48.29 and At 250 (mg/ml)

radial scavenginghas been estimated with a %IC of about 42.17 .The overall inhibitory concentration(IC) at 50mg/ml is 615.82. While, DPPH assay value for same concentration is 586.87. The antioxidant activity from aqueous methanol plant extracts has huge advantages over other biological entities especially because they do not employ cell cultures. Even though there are numerous literature reports regarding the radial scavenging much more plant extracts are undergoing research as potential candidates for the reducing agents as well as anti-oxidatating agent.

ACKNOWLEDGEMENT

The authors are privileged to thank our H. o. D. Dr. N. G. Ramesh Babu, Department of Biotechnology, Adhiyamaan College of Engineering (Autonomous), and Mr. Ganesh, Director, Alpha Omega Hi-Tech Bio Research Centre, Salem, for their encouragement and support during the work. We are very thankful to them.

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