

Original Article

ESTIMATION OF ALKALOIDS AND PHENOLICS OF FIVE EDIBLE CUCURBITACEOUS PLANTS AND THEIR ANTIBACTERIAL ACTIVITY

BIKASH DEBNATH^{b,c*}, MD. JASHIM UDDIN^a, PRASENJIT PATARI^c, MANIK DAS^b, DEBASISH MAITI^{a,c}, KUNTAL MANNA^b

^aDepartment of Human Physiology, ^bDepartment of Pharmacy, ^cState Biotech Hub, Department of Human Physiology, Tripura University (A central University) Suryamaninagar, West Tripura 799022
Email: bikashrips2014@gmail.com

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ABSTRACT

Objective: Objective of the present work was qualitative and quantitative estimation of alkaloids and phenolics of five edible cucurbitaceous plants and to evaluate their antibacterial activity against some human pathogenic bacteria.

Methods: Total alkaloid present was determined by acid-based titrimetric methods using methyl red as an indicator and observing a faint yellow end point. Total phenolics were estimated by follin-ciocalteu's method using tannic acid as standard. Antibacterial activity was determined by Disc diffusion method using SRL Agar medium. The 70% ethanolic dried powdered was dissolved in 20% DMSO at different concentration to carry out the anti-microbial activity.

Results: It was found that all the experimental plants contained almost equal amount of alkaloids but their phenolic contents as tannic acid equivalents were different. Alkaloids content of five Cucurbitaceous plants were found to vary from 1.15 g % to 1.34 g % and phenol content was varied from 4.54 mg/g to 10.13 mg/g. All the selected Cucurbitaceous plants were active against the tested pathogens, except against *V. cholerae non.0139 (L4)*. Only the 70% ethanolic leaf extract of *Momordica charantia* (Linn.) showed a relative percentage inhibition from 15.02 to 16.63. So, *Momordica charantia* (Linn.) extract was the most active among five selected plants against the tested pathogens.

Conclusion: The activity might be due to the presence of alkaloids and phenols. However, the extent of activity or zone of inhibition was found varied for different extracts might be due to the difference in the constituents present in the plant extracts.

Keywords: Cucurbitaceous, Alkaloids, Phenolics, Antibacterial activity, Human pathogen.

INTRODUCTION

The use of plants and plant products as a source of medicines could be traced back from the beginning of human civilization. The herbal wealth of India and the knowledge of their medicinal properties have a long tradition, as referred in Rig Veda and other ancient literature [1]. The Ayurveda has emphasized the importance of food in the management of diseases. Many researchers have paid much attention towards the Cucurbitaceous family, because the fruits, seeds and vegetables are traditionally consumed in various ayurvedic preparations and confectionary. The Cucurbitaceous includes a large group of plants which are medicinally valuable. It is a family of about 130 genera and about 800 species distributed mainly in tropical and subtropical regions of the world. The plants of the family are collectively known as cucurbits [2-4]. The increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggests that in order to find pharmaceutical active compounds, a systematic study of plants is very important [5]. India is a developing country, low-income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infectious diseases [6].

Antimicrobial activities of plant origin have enormous therapeutic potential as antibiotic. The potential plant antimicrobial activities are due to the secondary metabolites such as alkaloids, steroids, resins, tannins, phenolics, flavonoids, and fatty acids which have a potential physiological rule on the body [1, 7]. Alkaloids are a large group of plant secondary metabolites. They are pharmacologically active organic compound present in plant kingdom synthesized from amino acid having basic or cationic property due to the presence of positively charged α -nitrogen in their heterocyclic ring [8, 9]. The alkaloids are poisonous in nature, but when used in small quantities, exert useful physiological benefits in animals and human [10]. The alkaloid rich fraction obtained from various parts of plants is able to inhibit growth of almost all the tested bacterial species. It reveals a broad spectrum antibacterial property of the plant extracts. But, a limited quantity of

alkaloids present in any edible part of a plant may yield the better antimicrobial activity with lesser side effects [11, 12].

Phenolics are a class of chemical compounds comprise an aromatic ring, bearing a hydroxyl group (-OH), synthesized by plants in response to ecological pressures such as pathogen and insect invasion [13-16]. Natural phenolic compounds are widespread in the plant kingdom. They are found in leaves, fruits, bark and wood and can accumulate in the large amounts, in particular, organ or tissue in the plants [17]. They can affect the growth and metabolism of bacteria, activating or inhibiting the microbial growth according to their constitution and concentration [18-20].

From survey of the literature, it was found that few studies have been carried out on qualitative and quantitative estimation of alkaloids and phenolics and their antibacterial activity in the context of plants available in Tripura, North-East India [21, 22]. Moreover, there is no study with plant species of an edible cucurbitaceous family dealing with their alkaloids and phenolics content and their anti-microbial activity. In this backdrop, the presence research work is an attempt to carry out qualitative and quantitative estimation of alkaloids and phenolics of five edible cucurbitaceous plants and to evaluate their antibacterial activity and the impact of edible plant extracts on bacteria was the novelty of the study.

MATERIALS AND METHODS

Plant material collection

Fresh leaves of selected Cucurbitaceous plants *Coccinia cordifolia* (Linn.), *Ligenaria siceraria* (Mol.), *Cucumis sativas* (Linn.), *Luffa actangula* (Linn.), *Momordica charantia* (Linn.) were collected from the North Tripura district, India. All plant parts were collected in winter season generally in the month of November and December 2014, and were identified with the assistance of Badal Kumar Datta, a plant Taxonomist and Professor at the Department of Botany, Tripura University.

Test microorganism

The four clinically isolated bacterial species were used for the study, *Escherichia coli*, *Vibrio cholera non. 0139 (L4)*, *Shigella dysenteriae 1*, *Streptococcus pneumoniae*. These bacterial strains were collected from National Institute of Cholera and Enteric Disease, Kolkata and Agartala Government Medical College, Agartala, for antibacterial assay.

Chemicals

Ethanol, n-butanol, Wagner's reagent, ferric chloride, Hydrochloric acid (HCl), sodium hydroxide (NaOH), methyl red, folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), tannic acid, SRL agar medium, clarithromycin disk, All the chemicals except ethanol and clarithromycin disk were obtained from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). Ethanol was purchased from Merck and clarithromycin disk was purchased from Bio-rad. All these chemicals used were of analytical grade and used as received without further purification unless otherwise stated.

Processing of the plants materials

The collected leaves were washed thoroughly with distilled water, cleaned and cropped into the pieces and air dried completely. Then the pieces were powdered in a grinder and passed through a sieve (mesh no. 40) in order to obtain the uniform size of the powdered material. These powder materials were used for further experiment.

Preparation of plant extracts for qualitative determination of alkaloids and phenolics

5 gm of each of the powdered plant materials were taken into 50 ml of 70% chilled ethanol in a beaker and vigorously stirred. The slurry was transferred into a reagent bottle. It was kept for overnight at room temperature. Then the slurry was centrifuged at 6000 rpm for 15 min and supernatant was used for preliminary screening of alkaloids and phenolics.

Preparation of plant extracts for quantitative determination of alkaloids

5 gm of powdered plant material was taken into 20 ml of n-butanol and vigorously stirred. The content was transferred into a reagent bottle. The slurry was kept overnight at room temperature. Then it was centrifuged at 6000 rpm for 10 min and the supernatant was made up to 50 ml with n-butanol.

Preparation of plant extracts for quantitative determination of phenolics

200 mg of each of the powdered plant material was taken and crushed in 5 ml of chilled 80% ethanol. The slurry was taken into a centrifuged tube. It was then centrifuged in an ordinary centrifugation machine in 6000 rpm in 10 min. It was taken out and the supernatant was transferred into a 10 ml measuring cylinder. The volume of the supernatant was made up to 5 ml with chilled 80% ethanol. The resulting solution was used for colour production.

Preparation of plant extracts for antimicrobial assay

10 g of each of the selected powdered plant materials was taken and crushed in 100 ml of 70% chilled ethanol. The slurry was taken into a reagent bottle. This was kept at room temperature for overnight. Then the slurry was centrifuged at 6000 rpm for 15 min and supernatant was collected in a petri dish at room temperature until the solvent was evaporated up to dryness. The powder obtained was collected in a microcentrifuge tube and stored at 4 °C for further experiment.

Test for alkaloids [23]

The prepared sample for qualitative determination of alkaloids was used to preliminary test the total alkaloids. 2 ml of extract was treated with 3-5 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100 ml of water] and observed for the formation of a reddish brown precipitate.

Test for Phenolic content [23]

The prepared sample for qualitative determination of alkaloids was used to preliminary test the total phenolic content. 2 ml of the extract was treated with few drop of aqueous 5% ferric chloride and formation of deep blue or black colour indicate presence of phenol.

Determination of total alkaloids by titrimetric methods [24]

Obtained supernatant of the plant sample was used for the determination of total alkaloids by titrimetric methods. 10 ml of the supernatant was taken into a 100 ml separating funnel. 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 min. This results in the solubility of alkaloids. The lower layer contains alkaloids neutralized with 0.1 (N) HCl and the upper layer contains n-butanol. 10 ml HCL portion was collected in a beaker and 2-3 drops methyl red was added to it, that turns the solution into slightly reddish colour. The contents of beaker were titrated against 0.1 (N) NaOH, till colour change changed from red to pale yellow. The neutralization point was determined. Same procedure was repeated triplicate.

The total amount of alkaloids was calculated by considering the following equivalent:

1 ml 0.1N HCl \equiv 0.0162 g alkaloid

Determination of total phenolic by Folin-ciocalteu's method

The total phenolics present in the plant extract were determined by using Folin-Ciocalteu colorimetric method based of oxidation-reduction reaction [25].

A stock solution of 1 mg/ml tannic acid was prepared in 80% chilled ethanol. It was then diluted ten times and used as working standard solution. From this stock, 0.1, 0.2, 0.3, 0.4, 0.5 ml of sample were taken into separate test tubes. Then 0.5 ml of Folin-Ciocalteu reagent and 1 ml of saturated sodium bicarbonate solution was added to each of the test tube. The volume of each of the test tube was made up to 5 ml with distilled water. Then the content of all the test tubes were incubated into a boiling water bath for 2 min exactly. The test tubes were cooled at room temperature and the absorbance of each of the content of the test tube was measured at 560 nm into a UV-VIS Spectrophotometer (Dynamica HALO DB-20) against the reagent blank. The calibration curve is shown in fig. 1.

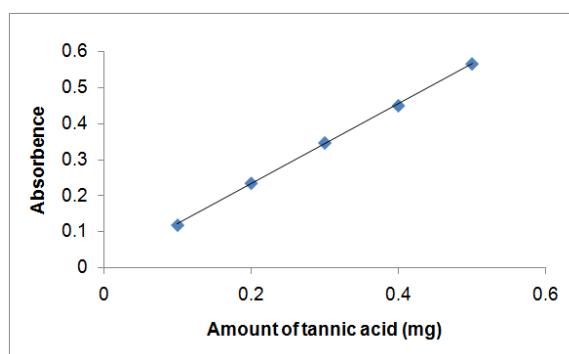


Fig. 1: Standard calibration curve of tannic acid

For samples, 0.4 ml of the extract prepared in material and method were repeated in triplicate format and the colour was generated as usual. The amount of phenol was calculated by the following way:

Each of the common factors was determined by dividing the absorbance value by the respective content. The average of five common factors thus obtained from five different concentrations was used. It was considered as the final multiplying factor. Then the amount of phenol was calculated by the following formula:

$$\text{common factor} \times \frac{1}{\text{Amount of plant material crushed (g)}} \times \frac{\text{Total amount of extract prepared (ml)}}{\text{Amount of extract taken (ml)}}$$

Antimicrobial assay by disc diffusion method [26, 27]

Already prepared powder plants sample was used to study the antibacterial assay by disc diffusion method. Three concentrations (250µg/µl, 375µg/µl and 500µg/µl) of those powder materials were prepared using 20% dimethyl sulphoxide (DMSO) as a solvent. The test microorganisms (freshly cultured) were seeded into the respective medium (SRL Agar) by spread plate method with 40 µl of each strain. After solidification of the agar, paper discs (5 mm in diameter and 0.4 mm in thickness), were placed in different Petri dishes (100 mm in diameter) containing agar media. Then 4 µl of each of the different concentrations of the extract was applied on each disc. 20% DMSO was also applied as a negative control for each concentration of the extract and clarithromycin (15µg/disc) a standard antibiotic disc place on the Petri disc as a positive control. After that, the plates were incubated at 37 °C into a 'Bacteriological Incubator' (N-Biotech NB-205Q) for 24 hr. Then the plates were taken out and the diameters of zone of inhibition of each disc were measured to evaluate antibacterial activity. All experiments were carried out in triplicate and the mean of the readings were recorded.

Determination of relative percentage inhibition [28, 29]

The relative percentage inhibition of the test extract was calculated with respect to positive control (clarithromycin 15µg/disc) by using the following formula:

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (X - Y)}{(Z - Y)}$$

Where,

X: Total area of inhibition of the test extract.

Y: Total area of inhibition of the negative control (20% DMSO).

Z: Total area of inhibition of the standard drug (Clarithromycin 15µg/disc).

The total area of the inhibition was calculated by using.

Area = πr^2 ; where, r = radius of zone of inhibition.

RESULTS AND DISCUSSION**Qualitative analysis of selected edible cucurbitaceous plants**

Phytochemical are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that biosynthesized them may have little need for them. They are naturally synthesized in all parts of the plant part such as bark, leaves, stem, root, flower, fruits, and seeds, etc. as active components [30]. Preliminary screening of active component is most important in pharmaceutical industry because screening is the first way for the development of a new pharmaceutical product like antibiotic, vaccine, sera, etc. [31, 32].

In the present study, a preliminary screening of five selected edible cucurbitaceous plants was carried out for qualitative analysis. Most of these are used by local people of Tripura in their daily life for nutrition purpose and cure the diseases. All of the plants selected were found to have alkaloids and phenols as active compounds. So, these five plant leaves had been taken for further analysis. The results of preliminary phytochemicals screening of five edible cucurbitaceous plant leaves are represented in table-1. Out of these 5 plants screened no plant was found to contain a high amount of alkaloids and phenolics. All of the 5 plants represent moderately high concentration (++) of alkaloids and 4 plants represent the moderately high concentration of phenol (++).

Table 1: Preliminary phytochemical analysis of selected edible cucurbitaceous family plants for presence of alkaloids and phenolics (+) refers to small concentration; (++) refers to a moderately high concentration

Local name	Botanical name	Family and plants part used	Alkaloids	Phenol
			Present (+)/Absence (-)	
Telachochi	<i>Coccinia cardifolia</i> (Linn.)	Cucurbitaceae (Leaf)	++	+
Lou	<i>Ligenaria siceraria</i> (Mol.)	Cucurbitaceae (Leaf)	++	++
Sosha	<i>Cucumis sativus</i> (Linn.)	Cucurbitaceae (Leaf)	++	++
Jhinga	<i>Lufa actangula</i> (Linn.)	Cucurbitaceae (Leaf)	++	++
Karela	<i>Momordica charantia</i> (Linn.)	Cucurbitaceae (Leaf)	++	++

Quantitative analysis of selected edible cucurbitaceous family plants [33-37]

The quantitative analysis of alkaloids and phenol present in the different parts of screened plants is represented in table-2. The range of total alkaloids content was found to vary from 1.15±0.01 g% w/v dry weights to 1.34±0.04 w/v dry weights and the range of total phenol content was found to vary from 4.54±0.29 mg/g w/v dry weight to 10.13±0.07 mg/g w/v dry weight equivalent to tannic acid. Among the samples analysed and comparative study of

alkaloids and phenol, *Ligenaria siceraria* (Mol.) was found to have the maximum amount of total alkaloid and *Lufa actangula* (Linn.) was found to have a minimum amount of total alkaloids. Besides, *Lufa actangula* (Linn.) was found to contain the maximum amount of phenol and *Coccinia cardifolia* (Linn.) was found to contain the minimum amount of phenol. The rest of the plants were found to contain moderate amount of alkaloids and phenol. It was very interesting to note that all of the selected plant belongs to the same family, thereby the content of alkaloids and phenolics are nearest to each other.

Table 2: Quantitative amount of total alkaloids and phenol in selected edible cucurbitaceous plants

Botanical name	Family and plant part used	Amount of total alkaloids (g% W/V) of dry weight	Amount of phenol (mg/g W/V TA) of dry weight
		(mean±SDs)	
<i>Coccinia cardifolia</i> (Linn.)	Cucurbitaceae (Leaf)	1.31±0.01	4.54±0.29
<i>Ligenaria siceraria</i> (Mol.)	Cucurbitaceae (Leaf)	1.34±0.04	9.96±0.15
<i>Cucumis sativus</i> (Linn.)	Cucurbitaceae (Leaf)	1.23±0.03	8.02±0.17
<i>Lufa actangula</i> (Linn.)	Cucurbitaceae (Leaf)	1.15±0.01	10.13±0.07
<i>Momordica charantia</i> (Linn.)	Cucurbitaceae (Leaf)	1.23±0.01	9.13±0.04

Anti-bacterial activity

Plants are being proved as an alternate source of therapeutic compounds. Natural products have been shown to be a potential

source of anti-infective agents; the classic example being that of Penicillin and Tetracycline. Alkaloids, phenolics, terpenoids, flavonoids etc are the class of natural compounds possessing a wide range of pharmacological activities [27, 38]. Antibacterial

activity of plants extract is due to the presence of different secondary metabolites [39].

The antibacterial activity of 70 % ethanolic leave extract (prepared as materials and methods) of five selected Cucurbitaceous plants against four human pathogenic bacteria are presented in table 4 and the activity of positive control and negative control against specific bacterial strains are represented in table 3. The results revealed that leaf extract of *Momordica charantia* (Linn.) showed significant antibacterial activity against *E. coli*, *S. dysenteriae 1*, *S. pneumoniae* (around 9.66-12.00 mm, the zone of inhibition). In the present study, it was found that *Cucumis sativus* (Linn.) showed comparatively less antibacterial activity rather than other plant extract studied.

Moreover, it was found from the literature that the content of any secondary metabolites (phytochemicals) and its activity as an antibacterial agent are different [40, 41]. That is why, *Cucumis sativus* (Linn.) and some others studied plant extract might be showing (table. 4) comparatively less antibacterial activity against specific pathogenic bacteria.

Relative percentage inhibition

The relative percentage inhibition (table 5) was evaluated by the formula mentioned in material and methods. *Momordica charantia* (Linn.) extract showed the significant relative percent inhibition among all of the bacterial strains.

Table 3: Measures (in mm) of zone of inhibition of positive control (Clarithromycin 15µg/disk) and negative control (20% DMSO) on four bacterial strains

Name of the pathogens	Inhibition zone diameter (mean±SDs)	
	positive control	Negative control
<i>E. coli</i>	21.33±1.15	0.00
<i>V. cholerae non.0139(L4)</i>	16.33±0.57	0.00
<i>S. dysenterae 1</i>	21.66±0.57	0.00
<i>S. pneumonia</i>	18.66±0.57	0.00

Table 4: Measures (in mm) of the zone of inhibition after 24 h, applying leaf extracts of selected cucurbitaceous plants on four bacterial strains. The '-' indicates no effect of extracts, whereas, the data showing zone of inhibition in mean±SD (disc radii= 5 mm)

Name of the pathogens	<i>Coccinia cardifolia</i> (Linn.)			<i>Ligenaria siceraria</i> (Mol.)			<i>Cucumis sativus</i> (Linn.)			<i>Lufa actangula</i> (Linn.)			<i>Momordica charantia</i> (Linn.)		
	1	15	2	1	15	2	1	1.5	2	1	1.5	2	1	1.5	2
	Conc. mg/disk zone of inhibition in mm														
<i>E. coli</i>	6.33±0.57	7.66±0.57	9.33±0.57	7.66±0.57	8.66±0.57	10.33±0.57	-	6.33±0.57	6.33±0.57	6.33±0.57	7.33±0.57	9.33±0.57	8.66±0.57	10.33±0.57	12.00±0.1
<i>V. cholerae non.0139(L4)</i>	-	-	-	-	-	-	-	-	-	-	-	-	6.33±0.57	6.66±0.57	6.66±0.57
<i>S. dysenterae 1</i>	6.33±0.57	8.33±0.57	8.33±0.57	7.33±0.57	8.33±0.57	9.33±0.57	-	6.33±0.57	6.33±0.57	6.33±0.57	8.33±0.57	8.66±0.57	9.66±0.57	10.33±0.57	11.33±0.57
<i>S. pneumonia</i>	6.66±0.57	8.66±0.57	9.66±0.57	6.33±0.57	8.33±0.57	8.33±0.57	-	-	-	6.66±0.57	8.66±0.57	9.66±0.57	9.66±0.57	10.33±0.57	11.33±0.57

Table 5: Relative percentage inhibition in different concentration of selected Cucurbitaceous Plants on four bacterial strains, The '-' indicates relative percentage inhibition is zero

Name of the pathogens	<i>Coccinia cardifolia</i> (Linn.)			<i>Ligenaria siceraria</i> (Mol.)			<i>Cucumis sativus</i> (Linn.)			<i>Lufa actangula</i> (Linn.)			<i>Momordica charantia</i> (Linn.)		
	1	1.5	2	1	1.5	2	1	1.5	2	1	1.5	2	1	1.5	2
	Conc. (mg/disk)% relative inhibition														
<i>E. coli</i>	8.78	12.90	19.14	12.90	16.49	23.47	-	8.78	8.78	8.78	11.81	19.14	16.49	23.47	31.68
<i>V. cholerae non.0139(L4)</i>	-	-	-	-	-	-	-	-	-	-	-	-	15.02	16.63	16.63
<i>S. dysenterae 1</i>	8.54	14.79	14.79	11.45	14.79	18.55	-	8.54	8.54	8.54	14.79	15.98	19.89	22.74	27.36
<i>S. pneumonia</i>	12.73	21.53	26.79	11.50	19.92	19.92	-	-	-	12.73	21.53	26.79	26.79	30.64	36.86

CONCLUSION

An acid based titrimetric method for estimation of alkaloids is a simple, less time consuming and rapid determination process. On the other hand, Folin-Ciocalteus' colourimetric method is also being a popular method for determination of total phenolics. The present study indicated that the experimental five Cucurbitaceous plants contain more or less equal amount of alkaloids, but their phenol contents were different. The content of total alkaloids and phenolics of some plants were more, but their antibacterial activity and relative percentage inhibition was found to be absent or low against the specific strain. This was due to the difference in the content of any secondary metabolites (phytochemicals) and its activity as an antibacterial agent. Very interestingly, it was observed that only the

70% ethanolic leaf extract of *Momordica charantia* (Linn.) showed a relative percent inhibition from 15.02 to 16.63. So, *Momordica charantia* (Linn.) extract was the most active among five selected plants against the tested pathogens.

Further investigations are on the way for successful specific isolation and purification of the alkaloids and phenolics to show their antibacterial activity in both *in vitro* and *in vivo* models.

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CONFLICT OF INTERESTS

Declared None

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