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Research Article

PHYTOCHEMICAL QUANTIFICATION AND AMYLASE INHIBITORY ACTIVITY OF *PIMENTA DIOICA*

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ABSTRACT

According to qualitative phytochemical analysis Saponins, Cardiac glycosides, Flavanoids, Alkaloids and Tannins were present in the aqueous alcoholic extract of *Pimenta dioica* leaves extract. Quantification of these constituents have been performed by spectrophotometric method and presented the results. Among these four constituents flavanoids are found to be in higher amount. Amylase inhibitory activity which will be an indicative about the antidiabetic action of the extract also performed by *in vitro* method and 88.89 % inhibition of amylase enzyme observed.

Keywords: Saponins, Cardiac glycosides, Flavanoids, Alkaloids, Tannins, Amylase enzyme.

INTRODUCTION

Pimenta dioica belongs to Myrtaceae family. Neal *et al*[1], described that *Pimenta dioica* known as all spice since it have mixed flavor of cinnamon, cloves and nutmeg. In 17th century Spanish explorers gave the name all spice due to its peppery flavor. It is an important component of pimento dram, a Jamaican alcoholic drink, and liqueurs such as Benedictine and Chartreuse. Ridley *et al*[2], reported that All spice is a digestive and has an antiseptic and slightly anesthetics action. Jenny *et al*[3], reported that a tibetan remedy PADMA 28 contain all spice as ingredient and the same is used for cancer treatment. Many scientists (Oussalah *et al*., 2007[4], Oussalah *et al*.[5], 2006 and Kamble *et al*.[6], 2008) reported that Pimento berry oil have good antimicrobial activity. According to park *et al*[7]. All spice berry oil has good nematicidal activity against the pinewood nematode. All spice berries found to be an effective antioxidant by the ferric thiocyanate method and oil stability index method. In our earlier studies we have reported that aqueous alcoholic leaves extract of Pimento do not have glycosides, but these have enriched cardiac glycosides; hereby making an effort to quantify the phytoconstituents present in all spice leaves. Amylase inhibitory effect of Pimento leaves extract also performed.

MATERIALS AND METHODS

Plants Materials

Healthy leaves, of the plant were collected from in and around area. The leaves were washed and dried in hot air oven at 30^0 C and milled in a mixer grinder to coarse powder.

Quantitative estimation of secondary metabolites

Estimation of Alkaloids

To 1 ml of ethanolic extract 5 ml phosphate Buffer at pH 4.7 was added then 5 ml BCG solution added; shaken the mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with atropine equivalents. [8]

Estimation of Flavanoids

Total flavanoid content was determined by Aluminum chloride method. 1 ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 minutes 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10 % Aluminium chloride was added. After 6 minutes incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. [8]

Estimation of cardiac glycosides

1 g of the sample treated with 10 ml of 70 % alcohol and purified by lead acetate Na_2HPO_4 solution before the addition of freshly prepared Baljet reagent. Soak overnight intensity of the colour produced is measured using spectrophotometer at 405 nm. Blank was carried out by water and Baljet reagent. [9]

Estimation of Saponins

1 ml of the test sample was dissolved in 80 % methanol, 2 ml of Vanillin in ethanol was added, mixed well and the 2 ml of 72 % sulphuric acid solution was added, mixed well 000 reagent blank. [8]

Determination of Tannins

100 mg of the sample was extracted with 5 ml of 80 % ethyl alcohol and centrifuged at 2000 rpm. The supernatant was taken for assay. One ml Folin–Cathechu Reagent was added to 1 ml of the alcoholic extract of the sample. 2 ml of 20 % sodium carbonate was added and heated for 1 minute. After cooling, the solution was made up to 10 ml with distilled water. A blank was prepared by adding all the reagents except the sample. The absorbency was read at 650 nm using spectrophotometer. [10]

Amylase Inhibitory activity

Alpha amylase hydrolyses alpha 1, 4-linkages of starch molecules in a random manner. The reducing sugars (mainly maltose) produced by the action of alpha- amylase react with dinitrosalicylic acid and reduce it to a brown/orange –red coloured product, nitroaminosalicylic acid. The starch hydrolyzed product concentration under a specified level of alpha-amylase enzyme, with and without inhibitor is used to express the alpha amylase inhibitory activity. The percentage inhibition was calculated by the equation given below[11]

$$\% \ \alpha\text{-amylase Inhibitory activity} = 100 - \left[\frac{[\text{Maltose}]_{\text{sample}} \times 100}{[\text{Maltose}]_{\text{control}}}\right]$$

Materials

- **Starch solution:** Take 1 g of potato starch and dissolved in 100 ml of 0.02 M phosphate buffer of pH7.
- **Dinitro salicylic acid reagent**: It can be prepared by dissolve at room temperature 1 g of 3, 5-dinitrosalicylic acid in 20 ml of 2N NaOH, add 50 ml of distilled water followed by 30 g of Rochelle Salt make the volume up to 100 ml with distilled water. Protect this solution from CO_2 and store at 4^0C.
- **Alpha-amylase enzyme solution:** Dissolve 6 mg of alpha amylase in 200 ml of 0.2 M phosphate buffer (pH 7) containing 0.006 M NaCl. From this stock solution take 10 ml, dilute to 100 ml with same buffer solution. The final concentration of enzyme in the solution is 30 µg/ml.
- **Maltose standard solution:** Dissolve 50 mg of maltose in 50 ml distilled water and store at 4^0C.
- **NaOH (4.5 %);** Weigh 4.5 g of NaOH, dissolves in approximately 80 ml distilled water, and make the volume up to 100 ml with distilled water.
- **NaOH (2N);** Weigh 8 g NaOH, dissolve in approximately 80 ml distilled water, and the final volume up to 100 ml with distilled water.
- **Phosphate buffer (0.2 M, pH 7):** Take 39 ml of 0.2 M, monobasic sodium phosphate solution and mix with 61 ml of 0.2M dibasic sodium phosphate solution and dilute to a total volume of 200 ml.
- **Phosphate buffer (0.02 M, pH 7)**; Take 10 ml of the above phosphate buffer (0.2 M) and dilute it to 100 ml with distilled water.

Preparation of maltose calibration curve; Pipette aliquots of 0.1 to 1.0 ml of maltose (100-1000 µg) solution into test tubes and make up the volume to 1 ml with suitable addition of distilled water. To each tube add 2 ml of dinitrosalicylic acid reagent. Cover tubes with marbles. Keep the tubes in water bath for 10 minutes. Cool the tubes and add 10 ml of distilled water to each test tube. The orange red colour formed is measured at 540 nm against a reagent blank.

Determination of α – Amylase inhibitory activity

- Pre incubate the entire reagents for 15 minutes at 37^0 C in a water bath.
- Pipette 0.5 ml of 1 % starch solution; add it to 0.25 ml of phosphate buffer (0.2M, pH 7) and 0.25 ml of α amylase enzyme solution.
- Similarly a second set of test tubes (blank) by using phosphate buffer in place of enzyme solution. Prepare a third set of test tubes containing 0.5 ml of starch solution, 2 ml of dinitro salicylic acid reagent. 0.25 ml of α-amylase enzyme solution; this set is called the zero time control.
- Incubate all the tubes at 37^0C for three minutes. At the end of the incubation add 2 ml of dinitro salicylic acid reagent to first and second set of tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes.
- After cooling under cold water, add 10 ml of distilled water; mix thoroughly and take absorbance at 540 nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve.
- One unit of enzyme activity is defined as that amount which liberates 1 µmol of reducing sugars (calculated as maltose) /min from soluble starch at 37^0C, pH 7, and under the specified experimental condition.

Preparation of extract and quantification of α- amylase inhibitor activity

- Take 1 g of sample and extract with 75 ml of distilled water and 75 ml of ethanol for 2 h, at 40^0C.
- Centrifuge the suspension at 5000 rpm.Collect the supernatant. Take 0.25 ml and incubate with 0.25 ml of enzyme solution for 15 minutes at 37^0C.
- Incubate all the reagents also at 37^0C for three minutes. At the end of the incubation add 2 ml of dinitro salicylic acid reagent to first, second and sample tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes.
- After cooling under cold water, add 10 ml of distilled water mix thoroughly and take absorbance at 540 nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve.
- One unit of enzyme activity is defined as that amount which liberate1µmol of reducing specified experimental condition.

RESULTS AND DISCUSSION

From the data obtained from Figure 1, we could find out that flavanoids are in higher concentration in *Pimento diocia* that was found to be 32.10 %. Other constituents are cardiac glycosides (20.37 %), saponins (20.56 %), alkaloids (22.56 %) and tannins (7-27 %). The maltose calibration curve was plotted, from the graph the concentration at which the sample absorbance value intercepts are taken as the appropriate sample concentration. The percentage α-amylase inhibition of *Pimento diocia* was found to be 86.66 %. Because of this high α-amylase inhibitory activity this compound may have anti diabetic activity and this compound have a wide applications in future research field.

Table 1: The estimated amount of phytoconstituents in *Pimento diocia* is as follows

S. No.	Name of Phytoconstituent	Total contents present (%)
01	Flavanoids	32.59 %
02	Alkaloids	22.56 %
03	Saponins	20.97 %
04	Cardiac glycosides	20.37 %
05	Tannins	7.27 %

Table 2: Maltose calibration curve values

CONCENTRATION	ABSORBANCE
0.1 ml	0.2317
0.2 ml	0.2664
0.3 ml	0.3045
0.4 ml	0.3459
0.5 ml	0.4015
0.6 ml	0.4379
0.7 ml	0.5016
0.8 ml	0.5563
0.9 ml	0.6125
1 ml	0.7345

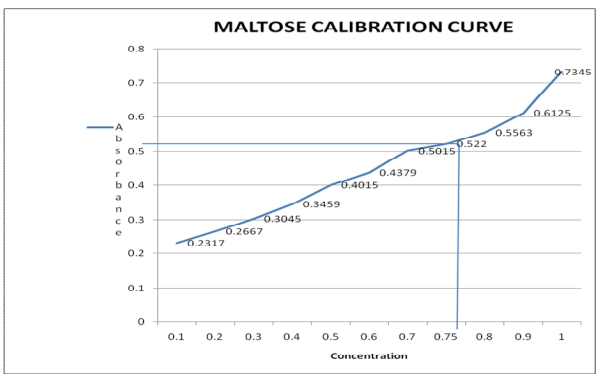

FIGURE 1

TABLE 3: Alpha-amylase inhibitory activity detection

Sample	Absorbance
Set 1 (E + S)	0.1452
Set 2 (blank)	0.2630
Set 3 (E + S + DNS)	0.3923
Extract (E + S + DNS)	0.5226

Where E = enzyme, S = starch, DNS = dinitro salicylic acid reagent

CONCLUSION

Qualitative phytochemical analysis has been performed in aqueous alcoholic extract of *Pimenta dioica*; then quantitatively estimated the amount of phytoconstituents using appropriate methods. Amylase inhibitory activity of the *Pimenta dioica* leaves extract also carried out according to the Bernfeld method. % inhibition of Amylase enzyme is found to be 86.66 %. This indicates that *Pimenta dioica* posse's inhibitory action on Amylase enzyme. In future studies phytoconstituents will be separated and will find out which constituent will be responsible for exhibiting the anti diabetic property.

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REFERENCES

1. Priya S Rao, Sheth Navinchandra, KN Jayaveera. An Important Spice, *Pimento diocia* (Linn) Merill; A Review International current Pharmaceutical Journal 2012; 1(8): 221-225.
2. Jenny M, Schwaiger W, Bernhard D, Wrulich O, Cosaceanu D, Fuchs D and Ueberall F. Apoptosis induced by the Tibetan herbal remedy PADMA 28 in the T Cell-derived lymphocytic leukemia cell line CEM-C7H2. Journal of Carcinogenesis 2005; 4: 15. http://dx.doi.org/10.1186/1477-3163-4-15
3. Oussalah M, Caillet S, Lacroix LSM. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E coli* 0157: H7, *Salmonella typhimu- rium*, *Staphylococcus aureus* and *Listeria monocytogenes*. An International Journal of Ceon Biblioteka Nauki 2007; 18: 414-420.
4. Oussualah M, Caillet S, Saucier L, Lacroix M. Antimicrobial effects of selected Plant essential oils on the growth of *Pseudomonas putida* strain isolated from meat. Meat Science 2006; 73: 236-244. http://dx.doi.org/10.1016/j.meatsci.2005.11.019
5. Kamble Vilas A and Patil SD. Spice derived essential oils: Effective antifungal and possible therapeutic agents. Journal of herbs spices and medicinal plants 2008; 14(3): 129-143. http://dx.doi.org/10.1080/10496470802598677
6. Park IK, Kim J, Lee SG, Shin SC. Nematicidal Activity of Plant Essential Oils and Components from Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*) and Litsea (*Litsea cubeba*) Essential Oils against Pine Wood Nematode (*Bursaphelenchus xylophilus*). J Nemato l 2007; 39(3): 275-279.
7. Narendra Devanaboyina, N Rama Lakshmi B *et al.* The International Journal of Science Inventions Today 2013; 2(1).
8. El Olemy MM, FJ Al Muhtadi and AFA Afifi. Experimental Phytochemistry: A laboratory manual, King Saud University Press. Saudi Arabia; 1994. p. 21-27.
9. Shanthi P Amuda. International Journal of Pharma and bioscience 2010; 1(4).
10. Bernfeld P. Amylases a and b in Methods in Enzymology, 1, Colowick S and Kapla N, eds, Academic Press, NY; 1955. p. 149.

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