Preliminary Phytochemical Investigation and Antioxident Activity of leaves from *Carica papaya* Linn; Thin-Baw and leaves of *Dolichandrone spathacea* (L.f) Tha-khut

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Abstract

This project is concerned with the phytochemical investigation of leaves of *Carica papaya* Linn; Thin-Baw and leaves of *Dolichandrone spathacea* (L.f) Tha-khut by test tube and TLC methods. The preliminary phytochemical tests of the selected plants revealed the presence of amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannin and terpenoids. The phytochemical constituents of petroleum ether extracts and 70% ethanol extracts were confirmed by thin layer chromatography method. From the observation, petroleum ether extracts from *Carica papaya* may contain steroids, terpenoids and alkaloids and ethanol extracts were found to be present terpenoids. Besides, the antioxidant activity of petroleum ether extracts and 70% ethanol extracts from the leaves of Thin baw and Tha-khut plants were measured by using1, 1 – diphenyl-2- picrylhydrazyl (DPPH) radical. All of 70% ethanol extracts from the leaves showed higher antioxidant potential than any other petroleum ether extract.

Keywords: *Carica papaya* L., *Dolichandrone spathacea* (L.f), phytochemical constituents thin layer chromatography, antioxidant activity, DPPH

INTRODUCTION

Photochemistry is the branch of science that deals with the study of phytochemicals. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occured chemical compounds found in plants, which provide benefits for human health further than those attributed to macronutrients and micronutrients. They protect plants from diseases and damage as well as contribute to the plant's colour, aroma and flavour. (Saxena *et al*., 2013)

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Carica papaya belongs to the family of Caricaceae. It is not a tree but a herbaceous succulent plant that possessed the supporting stem. The plants are usually short lived, but can produce fruit for more than 20 years. The plants are male, female and hermaprodite. The native of Papaya plant is tropical America. Now, the papaya is well known in nearly all tropical regions and the Pacific Islands. It is a rich source of three powerful antioxidants: vitamin C, vitamin A and vitamin E. It is used as a traditional medicine for the treatment of various diseases like cancer, malaria, dengue fever, viral infection such as common cold, eczema, warts etc. the studies conducted in some countries have shown that there is a significant antibacterial activity in organic extracts of different parts of *Carica papaya*. The leaf tea or extract of Papaya has a reputation as a tumour destroying agent. The high level of natural self defence compounds from the parts of the plant is highly resistant to insect and disease infestation (Wikipedia).

Dolichandrone spathacea (L.f) (Tha-khut) is one of the distributed plant species along the coastal region. It is known as Tui and mangrove trumpet tree. It is a species of the plants in Bignoniacea family. It can be found in the places from South India, Serilanka to New Caledonia.Mangrove Trumpet tree is an attractive, evergreen tree with intensely fragrant flowers, growing up to 20 meters the wild for local use as a food and medicine. The plant sample of *Dolichandrone spathacea* is a huge reservoir of variety of secondary metabolites like saponins, tannins, flavonoids, quinies , alkaloids, anthralene derivatives, reducing sugar, glycosides, carbohydrates, quercetin, kaempferol, iridoids, terpenes, steriods, etc.(Trease and Evans, 1980)

MATERIALS AND METHODS

Plant Materials

State in June the leaves of Carica papaya (Thin-Baw) and flowers and leaves of Dolichandrone spathacea (Tha-khut) were collected from campus of Taunggoke Degree College, Tounggoke Township, Rakhine, June, 2017.

***Carica papaya* (male & female)**

Dolichandrone pathacea

Figure 1. Selected Medicinal plants

Chemicals and Reagents

Chemicals used were petroleum ether, ethanol, ethyl acetate from BDH and also locally from the commercial chemical stores in Yangon, Silica gel 60 GF_{254} precoated aluminium sheets (20 cm x 20 cm) (Merck Ltd.,Japan) are used for TLC screening.

The reagents used for phytochemical tests were Dragendorff's, Mayer's, Wagner's, sodium picrate solution, 10%H_2SO_4 , 5%$FeCl_3$,10% ethanolic KOH, 1%$AlCl_3$,10% lead acetate, ninhydrin reagent,10% naphthol, conc.HCl, Mg turning,bromocresol green indicator, iodine solution, acetic anhydride, Benedict's solution and 1%gelatin.

Preliminary Phytochemical investigation of the Selected Medicinal Plants by Test Tube and TLC Methods

Preparation of PE and 70%Ethanol Extracts

Dried powder sample (30 g) was boiled with 150 mL of PE for about 30 minutes using Sohxlet extractor and then concentrated with water bath.

Dried powder sample (100 g) was percolate with 400 mL of ethanol in air tight container for two day at room temperature and then filtered and concentrated with water bath. The procedure was repeated for three times.

Procedure

Test for alkaloids

The dried powder sample (2 g) was treated in a test tube with 1% HCl (10 mL) for 30 min in a water bath. The suspension was filtered into a test tube and the filtrate was divided into three parts A, B and C (Marini-Bettolo, *et al.,* 1981). 5 drops of Wagner's reagent was added to filtrate A, 5 drops of Mayer's reagent was placed into filtrate B and 5 drops of Sodium picrate solution was added to the filtrate C.

Test for α-amino acids

The dried powder sample (2 g) was boiled with 25 mL of distilled water until 1/3 volume remained and filtered. The filtrate was then dropped on filter paper and sprayed ninhydrin reagent and heated in the oven at 90-100˚C for 10-15 minutes observation was made to see if violet color spots indicating presence of α-amino acid (Robinson, 1983).

Test for carbohydrate (Molish's Test)

The dried powder sample was boiled with distilled water and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-naphthol was added and shaken. The test tube was then inclined at an angle of 45˚ and concentrated sulphuric acid was added slowly along the side of the test tube. Red ring indicates the presence of carbohydrate (Shriner *et al*., 1980).

Test for flavonoids

The dried powder material (2g) was extracted with 70% ethanol and evaporated to dryness, and treated with 15 mL of pet ether (60-80˚C). A few drops of concentrated hydrochloric acid were added to the defatted alcoholic extract and then 0.5 g of magnesium ribbon was added. A colour which develops within 3 minutes indicates the presence of flavonoids. A pink, reddish pink, (or) brown colour was produced (Robinson, 1983).

Test for glycosides

The dried powder sample (3g) was boiled with distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10% lead acetate solution. White precipitate shows the presence of glycoside (Marini-Bettlo, 1980).

Test of phenolic compounds

The dried powder sample (3g) was boiled with distilled water and filtered. The filtrate when treated with three drops of freshly prepared (1:1) mixture containing 1% potassium ferricyanide and 1% ferric chloride solution. Observation was made to see if blue or green colour appeared (Robinson, 1983).

Test for saponins

Dried powder sample was introduced into a test tube followed by the addition of distilled water and the mixture was vigorously shaken for a few minutes. Observation was made to see if frothing took place (Shriner, *et al.*, 1980).

Test for steroids

The dried powder sample (3g) was refluxed with pet-ether and the solvent was removed by distillation under reduced pressure. Acetic anhydride (3 drops) was added and the mixture was shaken. Then a few drops of concentrated sulphuric acid were carefully added and shaken. Observation was made to see if the solution turned to blue colour (M-Tin Wa, 1972).

Test for terpenoids

The powdered sample (1 g) was extracted with chloroform (20 mL) for 30 minutes and filtered. There filtrate was evaporated to dry in desiccator and the residue was dissolved in ethanol (2 mL). The solution was transferred to watch glass and the solvent was evaporated to dryness on a water bath. The residue was dissolved in acetic anhydride, using a glass rod. The solution was treated with a drop of concentrated sulphuric acid. Observation was made to see brick-red colour (Vogel, 1956).

Test for tannin

Dried powdered samples 1g was boiled with distilled water 10 mL for about 20min and filtered. The filtrate was treated with a few drops of gelatin and 1% FeCl3. Observation was made to see if precipitate were performed (Marini-Bettlo, 1980).

Test for starch

Dried powdered samples 1g was boiled with distilled water 10 mL for about 30 min. It was then filtered and two drops of iodine solution was added to the filtrate. Observation was made to see if bluish-black precipitate were performed (Robinson, 1983).

Test of reducing sugars

1 g of dry powder sample was boiled with 10 mL of distilled water for about 20 minutes and filter. 1 mL of water extract was treated with 2 drops of Benedict's solution. Brick- red precipitate indicated presence of reducing sugars.(M.Tin Wa, 1972).

Test for organic acids

5 mL of water extract of the selected sample were taken and treated with a few drops of bromocresol green indicator. The appearance of blue colouration indicates the presence of organic acids. (Robinson, 1983)

RESULT AND DISCUSSION

Preliminary Phytochemical Investigation of Selected Plant Extracts by Test Tube Methods

In order to know the types of phytoconstituents, the phytochemical investigation was preliminarily carried out by test tube and TLC methods. According to the experimental results, alkaloids, $\propto -$amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar and steroids were observed in the selected plant leaves. However, starch, tannins, saponins and terpenoids were not observed in it. The results obtained from these experiments were summarized in Table (1).

Preliminary Phytochemical Investigation of Selected Plant Extracts by TLC Methods

In this work, TLC method was used for qualitative determination of phytoconstituents of PE and 70% ethanol extracts. The solvent system was optimized in order to get maximum separation on plate. The presence of various phytochemicals was detected by the use of different spraying reagents and visualized under UV light at 365 nm wavelengths. It was observed that steroids, terpenoids, alkaloids, essential oils and phenolic compounds were present in petroleum ether and 70% ethanol extracts of selected plants using solvent system (PE:EtOAC,1:1) and spraying agents (5% H_2SO_4, Δ, Mayer reagent). The results are shown in Figures 2 and 3.

Antioxidant Activity of Selected Plant Crude Extracts

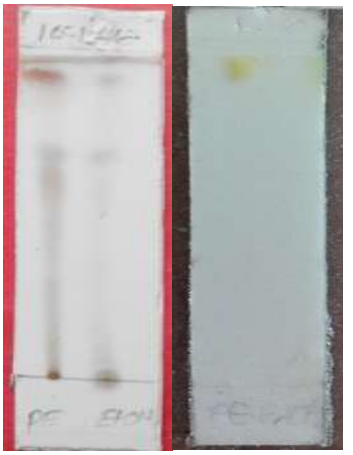
The antioxidant activities of crude extracts for leaves of selected plants were studied. Extracts of petroleum ether and 70% ethanol obtained were qualitatively determined by using DPPH- radical scavenging method.

According to the experiments, petroleum ether extract and 70% ethanol extract were investigated on its antioxidant activity. These extracts also showed antioxidant activity as shown in Figure 4 and 5.

Table 1. Preliminary Phytochemical Test on Selected Plant Extracts by Test Tube and TLC Methods

No	Test	Extract	Test Reagent	Observation	Leaves (Thin Baw)	Leaves (Tha khut)
1.	Alkaloids	1%HCl	Wagnar's reagent	Brown ppt	+	+
				Yellow ppt	+	+
			Sodium Picrate	Cream color ppt	+	+
2.	α-amino acid	H_2O	Ninhydrin reagent	Violet spot	+	+
3.	carbohydrates	H_2O	10%α-napthol +H_2SO_4	Red ring	+	+
4.	Flovanoids	EtOH	Mg ribbon, conc:HCl	Brown solution	+	+
5.	Glycoside	H_2O	10%Lead acetate	White ppt	+	+
6.	Phenolic compound	H_2O	1% $FeCl_3$ 1% K_3 $Fe(CN)_6$	Deep blue solution	+	+
7.	Recuding sugar	H_2O	Benedict's solution	Blue ppt	+	+
8.	Saponins	H_2O	H_2O	No frothing	-	-
9.	Starch	H_2O	1% I_2	No deep blue	-	-
10.	Steroids	PE	Acetic anhydride H_2SO_4	Deep blue	+	+
11.	Terpenoids	Chloro form	Acetic anhydride H_2SO_4	No pink colour	-	-
12.	Tannins	H_2O	1%$FeCl_3$,gelatin	No deep colour	-	-

(+) = present (-) = absent



a=petroleum ether extract, b=70%ethanol extract,

a b a b solvent system=PE:EtOAC,1:1,

Figure 2. Thin layer chromatograms of petroleum ether extract and 70% ethanol extract of *Carica papaya*

a=petroleum ether extract, b=70% ethanol extract,

c d c d solvent system=PE:EtOAC,1:1,

Figure 3. Thin layer chromatograms of petroleum ether extract and 70% ethanol extract of *Dolichandrone spathacea*

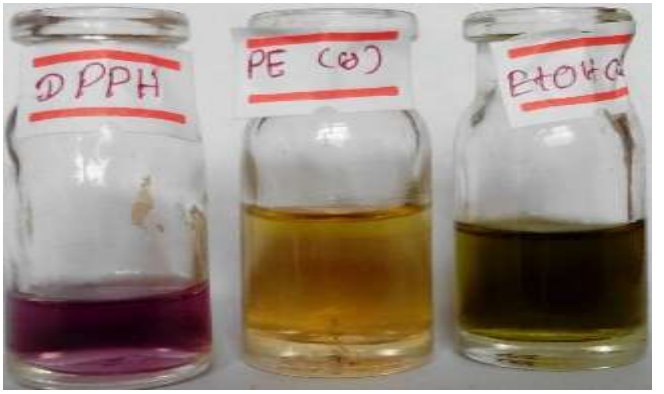
a b c

(a) Colour of DPPH

(b) Colour dimished after scavenging with pet ether extract

(c) Colour diminished after scavenging with 70% ethnol extract

Figure 4. Observation of antioxidant activity of pet- ether extract and 70% ethanol extract from *Carica papaya*



c d e

(c) Colour of DPPH

(d) Colour dimished after scavenging with petroleum ether extract

(e) Colour diminished after scavenging with 70% ethanol extract

Figure 5. Observation of antioxidant activity of petroleum ether extract and 70% ethanol extract from *Dolichandrone spathacea*

CONCLUSION

From the present work, the following inferences may be deduced.

The phytochemical tests indicated that the leaves of selected plants similarly contain alkaloids, α- amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, organic acids, steroids, tannins and terpenoids.

According to the screening by thin layer chromatography methods, chemical constituents of petroleum ether and 70% ethanol extracts from selected plants leaves may be steroids, terpenoids and alkaloids. Antioxidant activity examination of petroleum ether extracts and 70% ethanol extracts from selected plant leaves were also carried out by DPPH assay.

Significant antioxidant activity was observed in all extracts which showed scavenging DPPH radical. All petroleum ether extracts of selected plant leaves showed a weak antioxidant potential but all 70% ethanol extracts showed a great antioxidant activity.

ACKNOWLEDGEMENTS

I would like to thank Dr. Myint Swe, Principal of Taunggoke Degree College, for his permission to write this article in Taunggoke Degree College Research Journal. I would like to express Daw Khin Kay Thi Han and Daw Nyunt Nyunt Htay, lecturers, Depaitment of Chemistry, Tounggoke Degree College, for encouragement, helpful advice, and comments.

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