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Preliminary phytochemical analysis of *Dodonaea viscosa* leaves

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ABSTRACT

The aim of the present study was to investigate the presence of phytochemicals such as flavonoids, alkaloids, triterpenoids, saponins, tannins, reducing sugar, amino acid, steroids proteins and cardiac glycosides in the leaf extracts of selected medicinal plant, Dodonea viscosa collected from hills near Chengalpattu, Tamil Nadu, India. Acetone: Chloroform mixture (70:30) was used as solvents. Optimized solvent systems were used for thin layer chromatography (TLC) followed by contact bioautography to evaluate the bioactivities of the fractions.It was found that Alkaloids, tri terpenoids, amino acids, and cardiac glycosides were absent whereas flavonoids, saponins, tannins, reducing sugars and steroids were present in the extract. The TLC results indicates the presence of phytochemicals in the sample extract.

Keywords: Phytochemicals, *Dodonaea viscosa*, Acetone, Chloroform, Bioactive compounds

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INTRODUCTION

Studies on phytochemicals have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary & secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances. [1] Medicinal plants constitute the major constituents of most indigenous medicines and a large number of allopathic medical preparations contain one or more component(s) of plant origin. The medicines that are in use today are definitely not the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries have continuously contributed to the type, quality, presentation and concept of medicinal preparation. In the development of human knowledge for therapeutic use, scientists endeavored to isolate different chemical constituents from plants, subjected them to biological and pharmacological tests and then used them to prepare modern medicines [2]. There is increasing interest in the use of plant antioxidants for scientific research as well as for industrial (dietary, pharmaceutical and cosmetics) purposes. Significant activities of *Dodonaea viscosa* crude extract have been reported against gram positive, gram-negative organisms as well as a *C. albican* strain [3]. Anticandidal activity in crude acetone-based extract of the leaves of *D. viscosa* has been reported when forty clinical isolates of *C. albicans* including twenty each from HIV-positive and HIV-negative patients and a separate control strain, with MIC range of 6.25 to 25 µg/ml [4]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [5, 6]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [7, 8]. In the present work, qualitative phytochemicals analysis was carried out in *Dodonaea viscosa*, found in the hilly regions near Chengalpattu region in TamilNadu, India.

MATERIALS AND METHODS

2.1. Collection of samples

The medicinal plants used for the experiment were leaves of *Dodonaea Viscosa* collected from the hilly regions nearby Chengalpattu in TamilNadu

2.2. Preparation of extracts

500 grams of dried powder of *Dodonaea* leaves was packed in separate round bottom flask for sample extraction using mixture of two solvents namely 70% of acetone and 30 % of Chloroform. The extraction was conducted by 750 ml of the solvent mixture for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

2.3 Phytochemicals analysis

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature. [9, 10, 11]

2.3 a. Test for alkaloids

The extract of the crude dry leaf powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with equal amount of Dragondroff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids. [12]

2.3 b. Test for saponins

About 0.5 g of the plant leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

2.3 c. Test for tannins

About 0.5 g of plant leaf extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration. [13]

2.3 d. Test for steroids

2 ml of acetic anhydride was added to 2 ml of plant leaf extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

2.3 e. Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones. [14]

2.3 f. Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones. [14]

2.3 g. Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids. [14]

2.3 h. Test for Proteins

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicated the presence of peptide linkage of the molecule. [14]

2.3 i. Test for Amino Acids

To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple colour indicated the presence of amino acids in the sample. [14]

2.3 j. Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids. [14]

2.3 k. Test for Reducing Sugar

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates. [14]

2.4 Thin Layer Chromatography:

TLC Plate (from ready made-MERK)

A sample mixture was dissolved in solvent (Acetone) and spotted at one end of the silica gel plate. The plate was kept in the tank/beaker containing the mobile phase (Chloroform: Methanol – 19:1) in such a way that the end near the sample application should touch the mobile phase. Allow the chromatogram to run about 30 min. The plate was dried at 30-40°C at hot air oven. View the compound under UV Transilluminator.

Calculate the RF value. [15]

$$RF = \frac{\text{Distance moved by the solute (b)}}{\text{Distance moved by the solvent (a)}}$$

RESULTS

Table 1 shows that the phytochemicals constituents of *Dodonaea viscosa* extracted from the solvent of Acetone (70 %) – Chloroform (30%) mixture.

Sl No.	Phytoconstituents	Acetone– Chloroform Extract
1	Flavonoids	++
2	Alkaloids	--
3	Tri-terpenoids	--
4	Saponins	++
5	Tannins	++
6	Reducing sugar	++
7	Amino acid	--
8	Anthraquinones	--
9	Steroids	++
10	Proteins	--
11	Cardiac glycosides	--

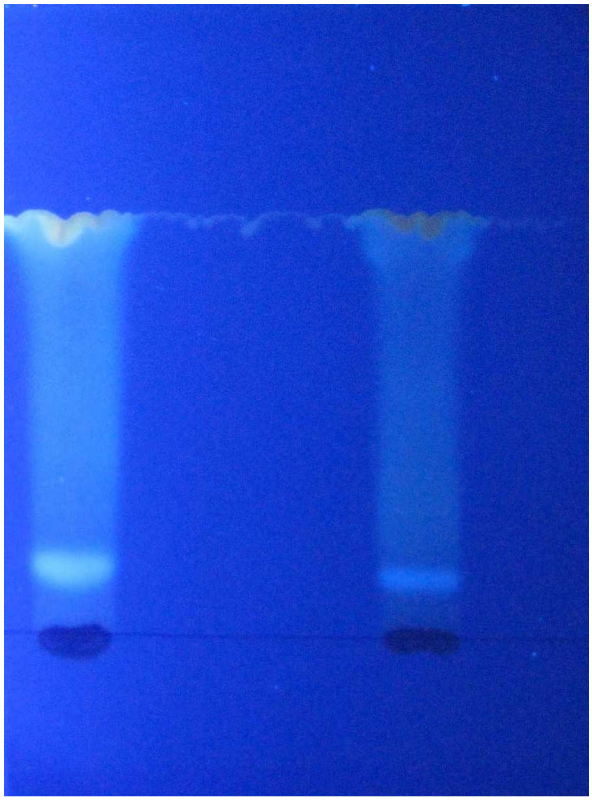
Positive: ++ Negative: --

Thin Layer Chromatography:

Table: 2

Sample	Sample travelling (cm)	Solvent front(cm)	Rf value
1	0.8	5.6	0.14
	5.6	5.6	1

Figure: 1



DISCUSSION

The study of the preliminary phytochemicals analysis revealed that these phytocompounds are mainly present in the acetonic solvent as shown in Table 1. The acetone extract of the samples of plant material were found to contain the required major phytocompounds and other nutritive compounds needed by the pharmaceutical companies as well as in food supplements. Further conforming the presence of phytochemicals in the sample, we performed the TLC and the report showed the bands (Figure: 1) and the RF value(Table: 2) indicating the presence of phytochemicals in the sample. Further analysis for individual components of the sample is in progress.

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