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Phytochemical analysis and antioxidant property of *Cadaba fruticosa*

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Abstract
Plants have been used for centuries as a remedy for human diseases because they contain phytochemical components of therapeutic values. Whole plant of the *Cadaba fruticosa* was selected to find out the phytochemicals and antioxidant property. Phytochemicals were extracted using various solvents such as Hexane, Ethyl acetate, ethanol and aqueous. Screening of phytochemicals showed positive results for the presence of carbohydrate in hexane, ethyl acetate, ethanol and aqueous extract, tannins in hexane, ethyl acetate, ethanol and aqueous extract, flavonoids in hexane, ethyl acetate, ethanol and aqueous extract, quinones in ethyl acetate, ethanol and aqueous extract, cardiac glycosides in hexane, Ethyl acetate, ethanol and Aqueous extract, terpenoids in hexane, Ethyl acetate and aqueous extract, phenols in hexane, Ethyl acetate, ethanol and aqueous extract and coumarins in hexane, ethyl acetate, ethanol and aqueous extract. Steroids in hexane and Ethyl acetate extract. Free radical scavenging activity was determined using DPPH is a stable antioxidant. Ethanol extract of *Cadaba fruticosa*, showed better antioxidant activity at a concentration of 75µg. These activities may be due to the presence of carbohydrate, tannins, flavonoids, terpenoids, phenols, coumarins and steroids.

Keywords: *Cadaba fruticosa*, DPPH, antioxidant, phytochemicals

Introduction
Cadaba fruticosa belongs to the family Capparaceae Indian Cadaba is a climbing shrub. The juice of the leaves is especially used to cure gonorrhoes.
Phytochemicals are defined as bioactive nonnutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases (Liu *et al.*, 2009) [12]. The phytochemicals vary in distribution within the plant parts, as well as in their occurrence within plant species. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Parr and Bolwell, 2008) [13]. Antioxidants are the compounds which help to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions (Jaleel *et al*., 2007) [9]. Free radicals contribute to more than one hundred diso rders in humans including atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Cook and Samman, 1996; Kumpulainen and Salonen, 1997) [6, 11] . Free radicals are often generated as by-products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented by Pourmorad *et al*., (2009). Medicinal plants can protect against harmful effects of ionizing radiation. Natural plant extracts or pure compounds are safe ingredients, which do not have any toxic effects. According to Kulkarni (1997) [10] plant extracts can be characterized by polyvalent formulations and interpreted as additive or in some cases potentiating. First the therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases (Feher *et al*., 1998; Aboutwerat *et al*., 2003) [7, 1]. Therefore, there is a need for isolation and characterization of natural antioxidants having less or no side effects, for use in foods or medicines to replace synthetic antioxidant. The present work deals with the preliminary phytochemical investigation of various extracts (hexane, ethyl acetate, ethanol and aqueous) of whole plant of Cataba fruticosa identify the major group of photochemical and also identification of free radical scavenging activity using DPPH assay.

Materials and Methods
Collection of Plants
Plants for this study were collected from Chinnapaliyapattu, Tiruvannamalai.

Preparation of Extracts
Collected plants were dried at room temperature and ground to make fine powder. 20gm of plant powder was well dissolved in 100ml of solvents (Hexane, Ethyl acetate and Ethanol) (ratio 1:5). The suspension was filtered by using Filter paper of pore size 0.2µm. The filtrate was then air dried and extracts were collected in sterile vials for further use.

Phytochemical tests
Test for carbohydrates
To 2ml of plant extract, 1ml of Molisch's reagent and a few drops of concentrated sulfuric acid were added. Presence of

purple or reddish color indicates the presence of carbohydrates.

Test for tannins
To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins
To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of a 1cm layer of foam indicates the presence of saponins.

Test for flavonoids
To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids
To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones
To 1ml of extract, 1ml of concentrated sulfuric acid was added. Formation of red color indicates presence of Quinones.

Test for glycosides
To 2ml of plant extract, 3ml of chloroforms and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides
To 0.5ml of extract, 2ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulfuric acid. The formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulfuric acid is added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for triterpenoids
To 1.5ml of extract, 1ml of Libemann –Buchard Reagent (aecticanhydride+ concentrated sulfuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

Test for phenols
To 1ml of the extract, 2ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for coumarins
To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Steroids and phytosteroids
To 1ml of plant extract equal volume of chloroform is added and subjected with a few drops of concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of the bluish brown ring indicates the presence of phytosteroids.

Phlobatannins
To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Anthraquinones
To 1ml of plant extract few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones.

Antioxidant activity
DPPH free radical scavenging activity
The ability of the extracts to annihilate the DPPH radical (1, 1-diphenil-2-picrylhydrazyl) was investigated by the method described by Blois (1958) [3]. A stock solution of leaf extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to the methanolic solution of DPPH (0.1mm). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula (Blos, 1958).

% of Inhibition = (A of control – A of Test) /A of control x 100

Results
The preliminary phytochemical screening of *Cadaba fruticosa* showed the presence of plant components such as carbohydrates, tannin, flavonoids, quinines, cardiac glycosides, terpenoids, phenols, coumarins and steroids in hexane extract, carbohydrates, tannins, flavonoids, quinines, cardiac glycosides, terpenoids, phenols, coumarins and steroids in ethyl acetate extract, carbohydrates, tannins, flavonoids, quinones, cardiac glycosides, terpenoids phenols, coumarins and steroids in ethanol extract and carbohydrate, tannin, flavonoid, quinines, cardiac glycosides, terpenoids and phenols in aqueous extract. (Table 1).

Table 1: Phytochemical analysis of *Cadaba fruticosa*

S. No	Phytochemical Tests				
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract	Aqueous Extract
1	Carbohydrates test	+	+	+	+
2	Tannins test	+	+	+	+
3	Saponins test	-	-	-	-
4	Flavonoids test	+	+	+	+
5	Alkaloid test	-	-	-	-
6	Quinones test	-	+	+	+
7	Glycosides test	-	-	-	-
8	Cardiac glycosides test	+	+	+	+
9	Terpenoids test	+	+	-	+
10	Phenols test	+	+	+	+
11	Coumarins test	+	+	+	-
12	Steroids and Phytosteroids test	+	+	-	-
13	Phlobatannins test	-	-	-	-
14	Anthraquinones test	-	-	-	-

Free radical scavenging activity

The stable free radical scavenging activity by the DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific plant extracts. Figure 2 indicates the percentage of free radicals scavenging activity in various extractions with different concentrations 25µg, 50µg and 75µg of *Cadaba fruticosa*. In this study percentage inhibition of free radicals was carried out with different extractions of selected plants. Ethanol extract with 75µg concentration gives higher percentage 73.30%, Hexane extract showed moderate activity of 47.57% and Ethyl acetate extract showed least activity of 38.13% of free radical scavenging activity. The free radical scavenging activity increases with increase in concentration(Table 2, Fig.1). The percentage inhibition of control was found to be 81.68% which showed higher activity than the extract.

Table 2: DPPH assay of *Cadaba fruticosa* against different extracts

Concentrations (µg)	Control	% of Inhibition			
		Hexane	Ethyl acetate	Ethanol	BHT
25	0.9593	11.05± 0.90	27.44± 1.03	61.66±1.20	72.36 ± 1.64
50	0.9593	43.26 ± 0.58	32.83± 0.53	68.13±0.68	76.23 ± 0.85
75	0.9593	47.57± 0.46	38.13± 0.48	73.30±0.43	81.68 ± 0.61

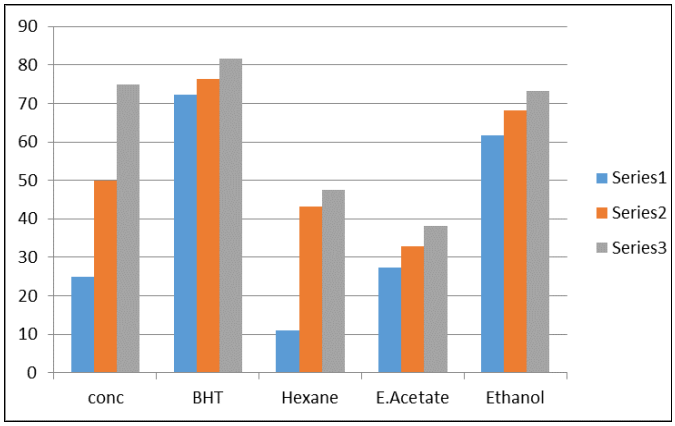

Fig 1: Antioxidant activity of *Cadaba fruticosa* by DPPH assay

Conclusion

The present study suggests that the *Cadaba fruticosa* have maximum number of bioactive components and higher amount of antioxidant potential in the ethanolic extract, therefore the ethanolic extract may act as a significant activity and can be further analysed for many pathogenic disorders as well as may be helpful in future for preventing or slowing the progress of diseases involved. However it is obvious that fewer information was available further in order to explore this plant more researchers should be carriedout.

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