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Phytochemical analysis and assay of antioxidant potentials of leaf extracts of *Cadaba fruticosa* and *Ocimum basilicum*

Juliya V Devasia[1], Sheba P T[1], Akhila George[1], Elsam Joseph[2]
[1] Research Scholar, Department of Botany, St. Teresa's College, Ernakulam, Kerala, India
[2] Associate Professor, Department of Botany, St. Teresa's College, Ernakulam, Kerala, India

Abstract
Medicinal plants have been used for centuries and are used as a potential source of therapeutic drug. The present study deals with phytochemical screening and antioxidant potentials of leaves of *cadaba fruticosa* and *Ocimum basilicum.* Phytochemicals were extracted using one polar and one non-polar solvent like Ethanol and chloroform. Varying amount of alkaloids, phenols, flavonoids, Terpenoids etc were detected. Preliminary phytochemical screening shows notable presence of Alkaloids and Phenols and its quantitative estimation showed alkaloid components are much high as compared to Phenolics in both the extracts. Ethanolic extracts showed very good antioxidant properties compared to chloroform extract. These results are highly significant as they can pave the way for future scientific validation of the traditional knowledge of these potential medicinal plants.

Keywords: medicinal plants, *Cadaba fruticosa*, *Ocimum basilicum*, physico chemical analysis, phytochemical analysis, anti-oxidant potentials

Introduction

Plant based medicines have been used from many centuries ago. Recently, herbal medicine are getting much popular than modern pharmaceutical medicines. With its prolonged use, safety, and efficacy, the herbal medicines have become a major concern for our society. Phytochemical screening of medicinal plants is therefore unavoidable for the development of new, efficient and targeted drugs. Reactive oxygen species play an important role in the cell signaling and homeostasis. The number of Reactive oxygen species increased due to some environmental stress and it is causing damages to cell structure which leads to cell death. Many medical conditions such as cancer, diabetes and anti-inflammatory disorders are accelerated due to the generation of Reactive oxygen species. These kinds of disease can be treated using herbal medicines. The bioactive principles of plants such as flavonoids, Phenolic components, tannins, alkaloids, saponins etc assured the efficiency of herbal medicine.

Cadaba fruticosa L. member of Capparaceae family is a climbing shrub commonly known as "vizhuthi" distributed mostly in the tropical and subtropical regions. In the Indian subcontinent *Cadaba fruticosa* was found in the Punjab, Central and western India, Gujarat, Tamil Nadu and Karnataka and have been utilized in Siddha, Ayurveda and other traditional systems of medicine for long. The plant grows up to 5 meters in height, bearing cylindrical stems.

The leaves are ovate, oblong, glabrous and fully margined. The leaves and fruits are used to treat worm infestation, swellings, eczema and constipation. The leaves contain 'cadabine' and also terpenoids flavones, sugar and proteins.

Phytochemicals are defined as bioactive non-nutrient plant compounds, distributed within the various plant parts. Scientific evaluation reveals that medicinal properties of *Cadaba fruticosa* includes antipyretic, anti-diabetic, antifungal, cytotoxic, antimicrobial, hepato protective and antioxidant properties.

Ocimum basilicum L. popularly known as 'king of herbs' belong to the family Lamiaceae, known for its therapeutic potential in traditional medicine. The plants have been used as a medicinal aromatic herb to add aroma and flavor to food. Basil is rich in various secondary metabolites like polyphenols, flavonoids and terpenes.

It is an annual plant producing white –purple flowers. Due to the characteristic flavors it imparts, the plant is used for culinary purposes. The plant grows well in many parts especially in the tropical regions of Asia, Africa and Central and South America. It contain many phytochemical compounds, gives various health benefits. Various studies showed that the plant Basil has Central (CNS) depressant, anticancerous, cardiac stimulant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulator, analgescic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, Chemomodulatory and larvicidal activities.

Material and Methods

Collection of plants

Cadaba fruticosa collected from Madhurai, and *Ocimum basilicum* from kuttampuzha, Kerala state. These plants were cleaned and washed in sterile distilled water and shade dried for 2-5 days. The dried plant species were powdered using blender and store for further use.

Physico- Chemical Parameters

The physicochemical characteristics of powdered leaves were determined as per the WHO guidelines.

Preparation of Leaves Extract

The fresh, undamaged and disease-free leaves were selected and washed thoroughly with sterile double distilled water (DDW), shade dried and then coarsely powdered in a blender. The coarse powder was soaked in 500ml of Chloroform and Ethanol solvents and kept in a shaker for 48hrs at 200 rpm. After 48 hrs it was filtered using whatsman No.1 filter paper. The extracts so obtained were further dried in vacuum desiccators. The residue obtained from the extract was used for further studies by preserving it in refrigerator.

Phytochemical Screening

The freshly prepared different leaves extract were qualitatively tested for the presence of chemical constituents. They identified by characteristic color changes and precipitation reactions using standard procedures. (Trease and Evans, 2009)

Determination of total phenolic content

Spectrophotometric methods are most commonly used for the quantification of phenolic content. Estimation of total phenolic content in the selected plant seed extract was measured spectro photometrically using Folin–Ciocalteu reagent.. Aliquots of 0.5 ml of the test sample and each sample of the standard solution were taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionized water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm wavelength. All the samples were analyzed in three replications.

Determination of total alkaloid content

Total alkaloid content was estimated based on the reaction between alkaloid and bromocresol green (BCG). The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. 1 ml of this solution was transferred to a separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm wavelength. The whole experiment was conducted in three replicates.

Antioxidant activity

DPPH (2, 2 –diphenyl – 1-picrylhydrazil) radicle scavenging activity

The ability of Leaf extracts to scavenge the DPPH (2, 2 –diphenyl – 1-picrylhydrazil) radicals was assessed by using the method of Blois with some modification (Blois, 1958). About 0.2 mol/L solution of DPPH in methanol was prepared, and 500 µL of this solution was added to different concentrations of the extracts (50-250 µg/mL). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Control was prepared as above but without the sample extracts and methanol was used for the baseline correction. Then changes in the absorbance of the plant samples were measured at 517 nm wavelength using spectrophotometer. A lower absorbance value indicates the higher radical scavenging activity. The ability of DPPH radical scavenging activity was calculated by using the following formula

% of inhibition = (Absorbance of control- Absorbance of Test)/ Absorbence of control*100

Results

Table 1: Comparison of Physico chemical parameters of *Cadaba fruticosa* and *Ocimum basilicum*

Samples	P^H value		Total Ash value (%)	Moisture Content (%)	Solubility test (%)	
	1%	10%			Water	Alcohol
C.fruticosa	5.42	5.51	17	4	24	34
O. basilicum	7.00	7.00	11.5	2	8	17

Table 2: Phytochemical Analysis of *Cadaba fruticosa*

Sl.no	Phytochemical test	Chloroform extract	Ethanol Extract
1	Saponins test	-	-
2	Flavonoids test	+	+

3	Alkaloid test	+	+
4	Terpenoids test	+	+
5	Phenols test	+	+
6	Tannins	+	+

(+) = Presence of Phytochemical, (-) = Absense of Phytochemical

Table 3: Phytochemical Analysis of *Ocimum basilicum*

Sl No	Phytochemical Tests	Chloroform extract	Ethanol Extract
1	Saponins Test	-	+
2	Flavonoids Test	+	+
3	Alkaloid test	+	+
4	Terpenoids test	+	+
5	Phenols test	+	+
6	Tannins	+	+

(+) = Presence of Phytochemical, (-) = Absense of Phytochemical

Table 4: Quantitative Phytochemical analysis

Sl.No	Plant extracts		Total alkaloid (%)	Total phenolics (%)
1	*Cadaba fruticosa*	E	2.04	0.15
		C	2.74	0.069
2	*Ocimum basilicum*	E	1.99	0.68
		C	4.1	0.14

E: Ethanol, C: Chloroform

Table 5: DPPH assay of of *Cadaba fruticosa* against different extracts

Sl. No	Ab.of Blank		Ab. of sample		Con. In PPM		Antioxidant activity	
	E	C	E	C	E	C	E	C
1	0.3477	0.3583	0.2241	0.3563	170.66	167.33	35.54±1.05	0.55±1.3
2	0.3477	0.3583	0.1521	0.3553	341.33	334.66	56.25±0.52	0.83±1.64
3	0.3477	0.3583	0.0942	0.3539	512	502	72.90±0.82	1.22±0.85

E: Ethanol, C: Chloroform

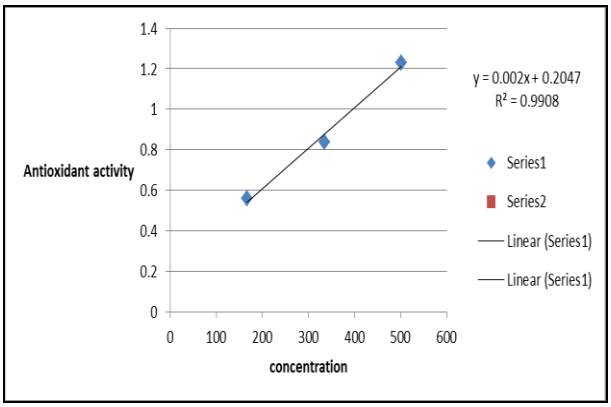

Fig 1: Antioxidant activity of *Cadaba fruticosa* (C)

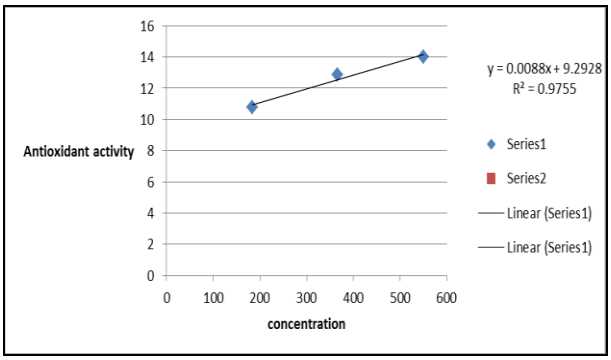

Fig 2: Antioxidant activity of *Cadaba fruticosa* (E)

Table 6: DPPH assay of of *Ocimum basilicum* against different extracts

Sl. No	Ab. of Blank		Ab. of sample		Con. In PPM		Antioxidant activity	
	E	C	E	C	E	C	E	C
1	0.3524	0.3608	0.2969	0.3306	172.66	160	15.74±1.03	8.37±1.64
2	0.3524	0.3608	0.2569	0.3156	345.33	320	26.24±0.53	12.52±0.82
3	0.3524	0.3608	0.2269	0.3023	518	480	35.61±0.43	16.21±0.65

E: Ethanol, C: Chloroform

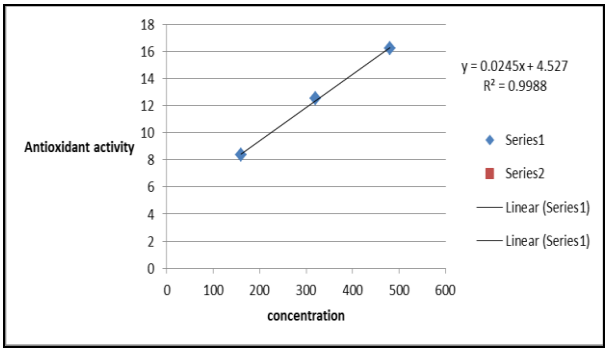

Fig 3: Antioxidant activity of *Ocimum basilicum* (C)

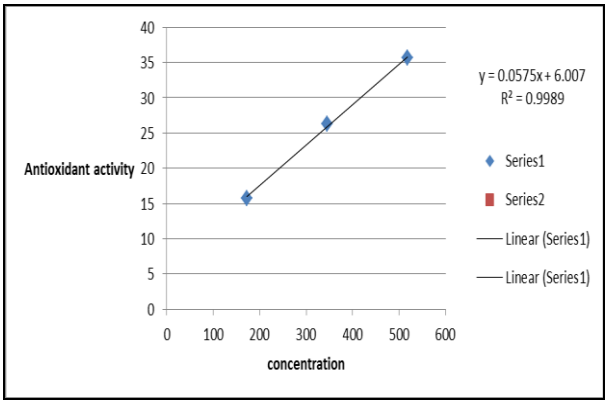

Fig 4: Antioxidant activity of *Ocimum basilicum* (E)

Discussion

The leaves of *Cadaba fruticosa* and *Ocimum basilicum* were collected and analyzed the various standardization procedures. The results of physico-chemical analysis of leaves of medicinal plants were represented in Table 1.The leaves of *Cadaba fruticosa* are slightly acidic in nature compared to the leaves of *Ocimum basilicum*, shows neutral p^H in both 1% and 10 % w/v aqueous solution. At Loss on drying at 105^0 C shows the presence of moisture content and also the presence of volatile oil in the drug. Total ash value of the material indicated the amount of mineral and earthy material attached to the plant material. (Meena *et al*., 2010.) [13]. The total ash value of leaves of *Cadaba fruticosa* and *Ocimum basilicum* are 17.5% and 11% respectively. Decadence time of the plant samples depends on the amount of the water present on it. If the water content is high, the plant can be easily vulnerable to microbial attack. The moisture retaining capacity of *Cadaba fruticosa* are high as compared to *ocimum basilicum.* The water soluble extractive values of leaves of *Cadaba fruticosa* (24.8%) and Ocimum basilicum (8.32%) indicates the possible presence of high polar compounds such as tannin, sugar, plant acid, mucilagea, and glycosides in the materials. The ethanol soluble extractive values are 34.56% and 17.61% respectively, show the presence of polar constituents like Phenol, alkaloids, Steroids and glycosides etc.

Phytoconstituent have been reported for anti-inflammatory, antidiarrheal, anti-microbial and antioxidant and insecticidal activities (Rashad *et al*., 2009).Preliminary phytochemical analysis showed the presence or absence of certain phytochemicals in the plant sample. The test was performed using both chloroform and ethanol extracts. The phytochemical analysis of five species are presented in Table 2& 3. On the six phytochemicals screened, Alkaloids, Flavonoids, Terpenoids, Phenols and Tannins are present in both the extract, except Saponin are present only in the ethanolic extract of *Ocimum basilicum*. Quantitative phytochemical analysis (Table-5)shows that these plants are rich source of alkaloids as compared to phenolics..Alkaloids are rich in chloroform extract of *Cadaba fruticosa* (2.74%) and *Ocimum basilicum* (4.1%).Total phenolics are higher in ethanolic extract of *Cadaba fruticosa* and *Ocimum basilicum.*

Antioxidants are mostly used for the protection of various diseases such as coronary heart disease, dementia, cancer, Alzheimer's disease, and arthritis (Moure *et al*., 2001). Antioxidant activity of ethanolic extracts by DPPH are recognizable as compared to Chloroform extract (Table 5& 6). % of inhibition of Ethanolic extract of

Cadaba fruticosa (72.90%) is high as compared to Chloroform extract (1.22%). % of inhibition of Ethanolic and Chloroform extract of *Ocimum basilicum* are 35.61% and 16.21% respectively. Half – maximal inhibitory concentration (IC50) the most widely used measurement for checking drug efficiencyIt indicated how much drug is needed to inhibit a biological process by half, the lower the IC50 value the more potent the drug (Aykul & Martinez-Hackert, 2016). The IC50 value of chloroform and ethanolic extracts of *Cadaba fruticosa* are 24.898mg/g and 5.0885mg/g respectively. IC 50 value of Chloroform extract (1.89mg/g) of *Ocimum basilicum* is high as compared to Ethanol extract (0.77mg/g). The results indicates that ethanolic extracts of *Cadaba fruticosa* and *Ocimum basilicum* shows much potent antioxidant activity compared to Chloroform extract.

Conclusion

Phytochemical analysis of the plants shows that the plants are rich source of various phytochemicals. The result of Anti-oxidant property produced by the ethanolic extracts of the plants *Cadaba fruticosa* and *Ocimum basilicum* was threshold of isolation of biomolecules from the natural sources in various drug development in the near future being responsible for the promising drug for pharmaceutical industry.

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