INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF *CLEOME VISCOSA* L.

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ARTICLE INFO

Article history
Received 16/04/2017
Available online
10/05/2017

Keywords
Cleome viscosa L.,
Phytochemical Analysis,
Antibacterial Activity,
DPPH Assay.

ABSTRACT

Cleome viscosa L. commonly known as Asian spider flower is used in Ayurvedic system of medicine for therapeutic purposes. The current study was undertaken with the aim of phytochemical analysis, evaluation of antibacterial activity and antioxidant properties of ethanol, acetone and methanol extracts of leaves this plant. The extracts were subjected to preliminary phytochemical analysis using standard phytochemical methods, *in-vitro* antibacterial activity against six standard bacterial strains by cup-plate agar diffusion method and antioxidant determination by DPPH method. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, phytosterols, fixed oil, saponins, phenolic compounds and flavonoids. Though all the extracts exhibited antibacterial activity, methanol extract of leaves registered highest antibacterial highest activity against *Staphylococcus aureus* (28 mm zone of inhibition), *Vibrio parahaemolyticus* (28 mm zone of inhibition), *Acinetobacter baumannii* (26 mm zone of inhibition), *Aeromonas hydrophila* (26 mm zone of inhibition), *Proteus mirabilis* (18 mm zone of inhibition) and *Yersinia enterocolitica* (15 mm zone of inhibition). The DPPH free radical scavenging assay revealed that the extracts exhibited scavenging effect in concentration-dependent manner. The strong free radical scavenging effect (43.33%) was observed with ethanol extract at 100 µg/mL which was comparable to that of standard ascorbic acid. This study confirmed the broad spectrum antimicrobial activity and free radical scavenging activities of *Cleome viscosa* L. and could be used as a potential alternative for treatment of various ailments.

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*Please cite this article in press as **Dr.C.Swaminathan**. Evaluation of Antibacterial and Antioxidant Properties of Cleome viscosa L.. Indo American Journal of Pharmaceutical Research.2017:7(04).*

INTRODUCTION

The rapid emergence of resistant microorganisms is occurring globally, endangering the efficacy of antibiotics, which have transformed medicine and saved countless millions of lives [1]. The crisis of antibiotic resistance has been attributed to the overuse and abuse of antibiotics, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [2]. Medicinal plant-derived compounds have increased widespread interest in the search of alternative antimicrobial agents because of the opinion that they are safe and have a long history of use in folk medicine for the treatment of infectious disease [3]. The main bioactive components in medicinal plants are considered to be combinations of secondary metabolites [4]. They are effective in the treatment of infectious diseases however, simultaneously mitigating many of the side effects that are associated with synthetic antimicrobial agents [5]. On the other hand, reactive oxygen species are produced by living organisms as a result of normal cellular metabolic activities and environmental factors [6]. Oxidative stress occurs when the balance between the reactive oxygen species and antioxidant defense system of the body is disturbed. Oxidative stress is an important contributor to various pathological conditions, including cancer, neurodegenerative diseases, arteriosclerotic vascular disease, hypertension, diabetes, inflammatory injury, drug toxicity, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma [7]. During pathophysiological conditions, there is an extra need for antioxidants from exogenous sources.

In view of the increasing trends of microbial resistance to antibiotics and negative health effects of synthetic antioxidants, there is a need for the development of safer therapeutic agents particularly from plants. *Cleome viscosa* L., (Cleomaceae) is a weed distributed throughout the tropics of the world and is known as wild mustard or dog mustard. The plant is reported to be useful in the treatment of malarial fever, head ache, otitis media, eye sores, ulcers, scurvy, rheumatism, skin diseases, gastrointestinal diseases, dyspepsia, bronchitis, liver diseases, uterine complaints and infantile convulsions [8-12]. The aim of this study was to evaluate the antibacterial and antioxidant activities of *Cleome viscosa* L. growing in Eastern Ghats of India which has not been subjected before to detailed study to reveal its antimicrobial and antioxidant potentials.

MATERIALS AND METHODS

Collection and Processing of Plant Materials

Fresh leaves of medicinal plant *Cleome viscosa* L. free from disease were collected from Shevaroy hills, Eastern Ghats, South India. After collection, leaves were removed, washed under running tap water and spread on mesh trays for shade drying. Upon drying, the leaves were grinded in to powder form and stored in air tight glass containers until required for analysis.

Preparation of Extracts

For alcoholic extraction 10 g of leaf powder was soaked separately in 100 mL of ethanol, acetone and methanol in a conical flask and kept for 48 hours at room temperature in laboratory shaker with a shaking speed of 120 rpm. The obtained extracts were filtered through Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till golden viscous residue was obtained and then stored at 4ºC for further analyses [14].

Preliminary Phytochemical Analysis

The crude ethanol, acetone and methanol extract of leaves of *Cleome viscosa* L. was tested for the presence of phytochemicals using the following qualitative procedures [13, 14].

Test for Carbohydrates

A small quantity of extract was dissolved separately in 4 mL of distilled water and filtered. The filtrate was combined with a small amount of Molisch's reagent in a test tube, after mixing, a small amount of concentrated sulphuric acid was added along the sides of the tube. Presence of carbohydrates is indicated by appearance of a purple ring at the interface between the acid and test layers.

Test for Reducing sugars

A small quantity of the extract was dissolved individually in 5 mL distilled water and filtered. The filtrate was subjected of Fehling's test and Benedict's test.

Fehling's Test

To 2 mL of Fehling's solution (1 mL of Fehling's A and 1 mL of Fehling's B solution), 2 mL of extract was added, mixed well and boiled. Appearance of yellow or red colour precipitate indicated the presence of reducing sugars.

Benedict's Test

5 mL of Benedict's reagent was added to 2 mL of extract and boiled for 3 minutes. Appearance yellow or red precipitate indicated the presence of reducing sugars.

Test for Alkaloids

A small portion of the alcoholic extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was subjected to Mayer's test, Dragendorff's test, Hager's test and Wagner's test.

Mayer's Test

The filtrate was treated with Mayer's reagent and the appearance of creamy precipitate indicated the presence of alkaloids.

Dragendorff's Test

A small amount of filtrate was treated with Dragendorff's reagent and appearance of orange red precipitate indicated the presence of alkaloids.

Hager's Test

The filtrate was treated with Hager's reagent and appearance of yellow colour precipitate indicated the presence of alkaloids.

Wagner's Test

The filtrate was treated with Wagner's reagent and the appearance of reddish-brown precipitate indicated the presence of alkaloids.

Test for Phytosterols

Salkowski test was done for the detection of phytosterols. The extract was dissolved in 2 mL of chloroform in a test tube. 1 mL of concentrated sulphuric acid was added along the sides of the tube and allowed to stand for 5 minutes. A reddish brown colour at the interface indicated the presence of phytosterols.

Test for Fixed Oil

Spot test was done for the detection of fixed oil. In this test, small quantity of alcoholic extract was pressed between two filter papers. Appearance of oil strain on the paper indicated the presence of fixed oil.

Test for Saponins

Froth test was done for the detection of saponins. Each extract was diluted with 20 mL distilled water and agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer 'honey comb' froth indicated the presence of saponins.

Test for Proteins and Free Aminoacids

Biuret Test

The plant extract was diluted with distilled water and treated with Biuret reagent. Appearance of pink or purple colour indicated the presence of proteins and free amino acid.

Ninhydrin Test

The diluted extracted was treated with ninhydrin reagent and observed for a characteristic purple colour which indicated the presence of free aminoacids.

Xanthoprotein Test

To 3 mL of the extract, 1 mL of concentrated nitric acid was added. The solution was heated for 1 minute and cooled under tap water. 40% sodium hydroxide was slowly added slowly until the mixture becomes alkaline and a colour change was noted. Change of colour from yellow to orange indicated the presence of aromatic aminoacids.

Test for Phenolic Compounds

A small quantity of the extract was dissolved in few mL of water and subjected to ferric chloride test. Each extract was dissolved in 5 mL of distilled water and a few drops of 5% ferric chloride solution was added. Appearance of bluish-black colour indicated the presence of phenolic compounds.

Test for Flavonoids

Alkaline reagent test was done for the detection of flavonoids. The extract was treated with a few drops of sodium hydroxide to give deep yellow colour. The disappearance of yellow colour after addition of dilute hydrochloric acid indicated the presence of flavonoids.

Bacterial Strains

Staphylococcus aureus (MTCC 3160), *Proteus mirabilis* (MTCC 743), *Yersinia enterocolitica* (MTCC 840), *Acinetobacter baumannii* (MTCC 9869), *Aeromonas hydrophila* (MTCC 1739) and *Vibrio parahaemolyticus* (MTCC 451) were procured from Microbial Type Culture Collection and Gene Bank, CSIR - Institute of Microbial Technology, Chandigarh, India.

Determination of Antibacterial Activity

The cup-plate agar diffusion method [14] was adopted to assess the antibacterial activity of the organic extract of the plant samples. The bacterial cultures were grown in Muller-Hinton broth (Hi-Media), adjusted to 0.5 McFarland turbidity standard and inoculated on Muller-Hinton agar (Hi-Media) plates by streaking the organisms over the surface of the medium using sterile cotton swab and allowed to dry for about 5 minutes. Cups of 6 mm in diameter were cut using a sterile cork borer and the cylindrical agar plugs were carefully removed. Using a micropipette, 100 µL of the alcoholic leaf extracts (100 mg/mL) dissolved in DMSO were poured in to appropriately labelled cups. 100 µL of nalidixic acid (500 µg/mL) and the blank solvent poured in to cups served as control. The plates were then kept at room temperature in upright position for two hours for diffusion of extracts and then incubated at 37ºC for 24 hours. Antibacterial activity was determined by measuring zone of inhibition around each well, averaged and the mean values were recorded.

Antioxidant Activity Determination by DPPH Radical Scavenging Assay

Free radical scavenging activities of solutions of the alcoholic leaf extracts and synthetic antioxidant substances used in the study prepared in methanol at concentrations of 10-100 µg/mL were determined in accordance with the Shimada method [15] which is based on the principle of scavenging the DPPH (2,2-Diphenyl-1-picryl-hydrazyl). DPPH was added to the solutions prepared with plant extracts and standard antioxidant substance ascorbic acid and stirred. Each mixture was kept in the dark for 30 minutes and the absorbance was measured at 517 nm against a blank. Percentage inhibition of DPPH activity was calculated using the formula given below.

$$\textbf{Percentage Inhibition} = [(A_0 - A_1 / A_0] \times 100$$

Where,

A_0 = Absorbance of control

A_1 = Absorbance of test sample or standard sample

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of ethanol, acetone and methanol extracts of leaves of *Cleome viscosa* L. revealed the presence of carbohydrates, alkaloids, phytosterol, fixed oil, saponins, phenolic compounds and flavonoids (Table-1). The presence of these phytochemicals provoked for an antibacterial and antioxidant studies on this plant.

Table-1: Phytochemical Screening of *Cleome viscosa* L. Leaf Extracts

S.No	Phytochemical Tests	Ethanol Extract	Acetone Extract	Methanol Extract
1	Carbohydrates: Molisch's test	+	+	+
2	Reducing Sugars Fehling's test	-	-	-
	Benedict's test	-	-	-
3	Alkaloids: Mayer's test	+	+	+
	Dragendorff's test	+	+	+
	Hager's test	+	+	+
	Wagner's test	+	+	+
4	Phytosterol: Salkowski test	+	+	+
5	Fixed oil: Spot test	+	+	+
6	Saponins: Froth test	+	+	+
7	Proteins and free amino acids Biuret test	-	-	-
	Ninhydrin test	-	-	-
	Xanthoprotein test	-	-	-
8	Phenolic compounds: Ferric chloride test	+	+	+
9	Flavonoids: Alkaline reagent test	+	+	+

'+' positive, '-' negative.

The antibacterial activity of ethanol, acetone and methanol extracts of *Cleome visocosa* L. leaves were tested against standard bacterial strains (Table-2). From the results, methanol extracts were the most effective and the highest activity was demonstrated against *Staphylococcus aureus* (28 mm), *Vibrio parahaemolyticus* (28 mm), *Acinetobacter baumannii* (26 mm) *Aeromonas hydrophila* (26 mm), *Proteus mirabilis* (18 mm), *Yersinia enterocolitica* (15 mm) followed by ethanol extracts with highest activity against *Aeromonas hydrophila* (15 mm), *Staphylococcus aureus* (13 mm) and *Acinetobacter baumannii* (11 mm). The acetone extract showed slight activity against test bacterial pathogens with the zone size ranging from 3 mm to 8 mm. The antibacterial activity of the methanol extracts of leaves of *Cleome viscosa* L. were higher than that of standard antibiotic nalidixic acid which is in agreement with the results obtained by Saradha and Subba Rao [16]. The presence of phytochemicals has been reported to confer antibacterial property to plant extracts [17, 18].

Table-2: Antibacterial Activity of Leaves of *Cleome viscosa* L.

S.No	Bacterial Pathogens	Diameter of Zone of Inhibition (mm)			
		Nalidixic Acid	Ethanol Extract	Acetone Extract	Methanol Extract
1	*Staphylococcus aureus* (MTCC 3160)	16	13	8	28
2	*Proteus mirabilis* (MTCC 743)	17	8	4	18
3	*Yersinia enterocolitica* (MTCC 840)	25	9	3	15
4	*Acinetobacter baumannii* (MTCC 9869)	24	11	7	26
5	*Aeromonas hydrophila* (MTCC 1739)	26	15	6	26
6	*Vibrio parahaemolyticus* (MTCC 451)	25	8	7	28

Radical scavenging activities are very important to prevent the harmful role of free radicals in different diseases, including cancer [19]. DPPH stable free radical method is one of the best method to determine the antioxidant of plant extracts [20]. The DPPH free radical scavenging of *Cleome viscosa* L. leaf extracts were evaluated and compared with ascorbic acid (Table-3). Ethanol extract of leaves showed highest radical scavenging activity. The results of the present study are in agreement with those reported by Pillai and Nair [21]. The strong free radical scavenging activity exhibited by the alcoholic extracts of leaves could be attributed to phenols and flavonoids. The results obtained in this study suggest that ethanol, acetone and methanol leaf extracts showed radical scavenging activity by their electron transfer or hydrogen donating ability.

Table-3: DPPH Free Radical Scavenging Activity of Standard Ascorbic Acid and *Cleome viscosa* L. Leaf Extracts

S.No	Concentration (µg/mL)	DPPH Scavenging Effect (%)			
		Ascorbic Acid	Ethanol Extract	Acetone Extract	Methanol Extract
1	10	26.16	25.19	21.42	22.61
2	20	27.50	27.16	22.22	24.69
3	30	30.15	30.06	24.65	27.05
4	40	32.10	30.50	25	30.13
5	50	33.18	32	26.08	30.20
6	60	40.09	37.31	27.12	31.34
7	70	43.18	39.06	31.25	32.81
8	80	44.07	41.66	33.30	40.50
9	90	50.05	42.39	37.05	41.10
10	100	51.50	48	40	43.33

CONCLUSION

In the present study, methanol extracts of leaves of *Cleome viscosa* L. showed very good antibacterial activity against a battery of bacterial pathogens responsible for skin infections, otitis media, urinary tract infection, wound infection, pneumonia and gastroenteritis. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, phytosterols, fixed oil, saponins, phenolic compounds and flavonoids. Ethanol extract of leaves of this plant showed highest free radical scavenging activity in a dose dependent manner. The obtained results could serve as a primary basis for further pharmacological studies in the discovery of potential natural bioactive compounds.

ABBREVIATIONS

DPPH : 2, 2-Diphenyl-1-picryl-hydrazyl
DMSO : Dimethyl sulfoxide

ACKNOWLEDGEMENT

The author thank the Chairman and Secretary of Vivekanandha Educational Institutions for Women, Tiruchengode, India for his encouragement and support.

COMPETING INTERESTS

The author declare no conflicts of interest.

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