Research Article

PHYTOCHEMICAL ACTIVITY OF AERIAL PARTS OF *CORDIA OBLIQUA* WILLD.

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Article Received on: 22/04/19 Approved for publication: 20/05/19

DOI: 10.7897/2230-8407.1007226

ABSTRACT

Most of the plant sources produce positive effect in human beings with recommended dose. Plants are rich in phytochemicals may help to prevent the body's defense against viruses, bacteria and other causing agents. Phytochemicals help to regulate hormones such as estrogen and insulin. Excess levels of these hormones increased the risk of breast and colon cancer. It inhibits inflammation, retard cell growth and works as a antioxidant, may aid immunity and boost production of detoxifying enzymes in the body. It blocks the carcinogens and prevent tumor growth. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. The aim of the study was to evaluate the phytochemical activity of ethanolic extract of aerial parts of *Cordia obliqua* Willd. Ethanol is the most common Bio-solvent obtained by the fermentation of sugar-rich materials such as sugar beet and cereals. The aerial parts were collected and extract prepared from ethanol by hot continuous percolation method in Soxhlet apparatus for 24 hours. The phytochemical screening results reveal the presence of tannins, steroids, alkaloids, flavonoids, phenolic compounds, triterpenes, saponins, gum and mucilage showed stronger protective action against life threatening diseases.

Keywords: Phytochemicals, Antioxidants, Ethanol, Plants, *Cordia obliqua*

INTRODUCTION

Phytochemicals are also called as plant chemicals, researchers have identified thousands of different phytochemicals found in Plant and plant derived natural products such as tannins¹, triterpenoids², flavonoids³, steroids⁴, carotenoids^{5, 6, 7}, polyphenols⁸, isoflavones⁹ and alkaloids may help to reduce oxidation, the damage to cells that occurs with aging and exposure to pollution, slow the growth rate of cancer cells like breast¹⁰, colon and prostate cancer¹¹, reduce inflammation, prevent DNA damage, stimulate immune system and reduce the risk of nervous system disorders, cardiovascular¹², genetic, and inflammatory diseases. The *Cordia obliqua* Willd. (clammy cherry) is a flowering plant species in the genus *Cordia* belonging to the family boraginaceae. It is found worldwide, mostly in warmer parts¹³ of India and Ceylon. There are 13 species found in India one of them is *Cordia obliqua* Willd. It is a medium sized deciduous tree and no heart wood¹⁴, leaves are alternate, flowers are bisexual, fruit is drupe and the seeds are mild sweet in taste. Traditionally *Cordia obliqua* is used for cooling effects, anthelmintic, expectorant and diuretic. It lessens thirst and scalding of urine, remove pains in the joints and it is used as treatment of diseases of spleen and leprosy^{15, 16}. Stem bark¹⁷ is used as a mild tonic, kernels are a remedy in treatment in ringworm and leaves are used to treat ulcers and head ache externally. Seeds are used as anti-inflammatory¹⁸ and antimicrobial agent¹⁹. A pickle is prepared from raw fruits of *Cordia obliqua* and also used as vegetable²⁰. The mucilaginous substance of the fruit can be used as a gum for pasting sheets of paper and cardboard.

MATERIALS AND METHODS

Collection and Identification of *Cordia obliqua*

*Cordia obliqua*²¹ was collected from B.Maduvangarai, Chidambaram Taluk, Cuddalore District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. (Reference: Flora of Tamilnadu, Volume 2, Page: 97, Year 1987)

Extraction and Isolation of *Cordia obliqua*

The aerial parts of *Cordia obliqua* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an air-tight container. The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus for 24 hours. The extract was concentrated by using a rotary evaporator²² and subjected to freeze drying in a lyophilizer²³ till dry powder was obtained.

Phytochemical screening of ethanolic extract of *Cordia obliqua*

The ethanolic extract was subjected to preliminary phytochemical screening^{24, 25} for the detection of various active constituents present. The term qualitative analysis refers to the constructing and providing the identity of a compounds. The pharmacological actions of crude drugs were determined by the nature of their constituents the phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used.

The ethanolic extract of aerial parts of *Cordia obliqua* was subjected to the following chemical identification tests were used for the detection of various active compounds.

Test for alkaloids

A little portion of the solvent free extracts were stirred individually with a mild drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents such as:

a) Mayer's reagent

A fraction of the extract was treated with Mayer's reagent and observed for the formation of white precipitate or cream coloured precipitate indicated the presence of alkaloids.

b) Dragendroff's reagent

A fraction of the extract was treated with Dragendroff's reagent and observed for the formation of yellow coloured precipitate indicated the presence of alkaloids.

c) Hager's reagent

A fraction of the extract was treated with Hager's reagent and observed for the formation of yellow coloured precipitate indicated the presence of alkaloids.

d) Wagner's reagent

A fraction of the extract was treated with Hager's reagent and observed for the formation of reddish brown precipitate indicated the presence of alkaloids.

Test for carbohydrates and glycosides

a) A small quantity of extract was dissolved separately in 5ml of distilled water and filtered. The filtrates were subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic α -naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of test tube, Appearance of purple or reddish violet ring at the junction of two layers shows the presence of carbohydrates. An another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate were subjected to Borntrager's tests and Legal's test to find out the presence of various glycosides.

b) Legal's test

To the hydrolysate, 1 ml pyridine and add a few drops of sodium nitroprusside solution and then it was made alkaline with the addition of sodium hydroxide solution. Facade of pink to red colour shows the manifestation of glycosides and aglycones.

c) Borntrager's test

A few ml of dilute HCl was added to 1 ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was separated and equal amount of dilute ammonia solution was added. Ammoniacal layer acquires rose pink colour shows the occurrence of glycosides.

d) Fehling's test

To 1 ml of extract and add equal amount of Fehling's solution A and B and boil for few minutes. A brick red precipitate indicated the presence of carbohydrates.

e) Benedict's test

To 5ml of Benedict's reagent with 1 ml of extract solution was added and boiled for 2 minutes, then cooled. Formation of red precipitate showed the presence of carbohydrates.

f) Baljet test

To 1 ml of ethanolic extract was added with 1 ml of sodium picrate solution. Yellow to orange colour revealed the presence of glycosides.

g) Keller killiani test

The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to test tube containing sulphuric acid. At the junction, formation of reddish brown colour, which gradually became blue showed the presence of glycosides.

Test for phytosterol

a) Liebermann Burchard test

Small amount of the extract was dissolved in few drops of chloroform and 3 ml acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of rose red colour to bluish green colour shows the presence of phytosterol.

b) Salkowski's test

Small quantities of the extract were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. Chloroform layer shows cherry red colour and concentrated sulphuric acid layer shows green fluorescence indicated the presence of steroid components.

Test for fixed oils and fats

a) Spot test

Small quantity of extract was separately pressed between two filter papers. oil stains on the filter paper indicates the presence of fixed oils.

b) Saponification test

Few drops of 0.5 N alcoholic potassium hydroxide was added to a small quantity of extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. The formation of soap or partial neutralization indicates the presence of fixed oils and fats.

Test for saponins

The ethanol extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 mts. A ^{1%} 1 cm layer of foam shows the presence of saponins.

Test for tannins and phenolic compounds

Small quantities of extract was dissolved separately in water and tested for the following tests:

a) Ferric chloride test

1 ml of extract was added with dilute ferric chloride solution (5%) and observed for the formation of a dark blue of greenish black colour indicates the presence of tannins and phenolic compounds.

b) Gelatin test

A fraction of the extract was treated with 1% solution of gelatin containing 10% sodium chloride and observed for the precipitation indicates the presence of tannins and phenolic compounds.

c) Lead acetate test

A fraction of the extract was treated with 10% lead acetate solution appears as white precipitate indicates the presence of tannins and phenolic compounds.

Test for proteins and free amino acids**a) Millon's reagent**

Small quantity of extract was dissolved in few ml of water and treated with Millon's reagent. Appearance of red colour shows the presence of proteins and free aminoacids.

b) Ninhydrin reagent

Small quantity of extract was dissolved in few ml of water and treated with Ninhydrin reagent. Appearance of purple colour shows the presence of proteins and free aminoacids.

d) Biuret test

1ml of extract was treated with equal volume of 40% sodium hydroxide solution and followed by a 2 drops of 1% copper sulphate were added. Appearance of pink to purple colour shows the presence of proteins and free aminoacids.

e) Xanthoproteic test

1 ml of extract was treated with 1 ml of concentrated nitric acid. A white precipitate is formed, it was boiled and cooled, then add 20% of sodium hydroxide or ammonia was subsequently added. Orange colour indicates the presence of aromatic amino acids.

Test for gums and mucilages

About 10ml of extract was added separately to 25 ml absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examines its swelling properties.

Test for flavonoids

Small quantities of extract were dissolved in few ml of water and treated;

a) With aqueous sodium hydroxide solution - yellow colour flavones.

b) With conc. Sulphuric acid - yellowish orange colour (Flavones)

c) Shinoda's test

The extract is treated with 5ml of 95% ethanol, to that 0.5 gm of magnesium turnings followed by concentrated hydrochloric acid in dropwise were added and heated. Appearance of magenta or pink colour shows the presence of flavonoids.

f) Alkaline reagent test

An aqueous solution of extract is treated with 10% of ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

g) Magnesium and hydrochloric acid reduction

A fraction of extract is dissolved in 5ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid are added. Pink to crimson colour indicates the presence of flavonol glucoside is inferred.

Test for lignin

Small quantities of extract was dissolved in few ml of alcohol and treated with alcoholic solution of phloroglucinol and hydrochloric acid gives red colour shows the presence of lignin.

Test for triterpenoids

Two or three granules of tin metal in 1ml thionyl chloride solution were dissolved 1ml of the extract was added into a test tube. The formation of pink colour indicates the presence of triterpenoids.

Test for coumarins

Moistened plant extract (0.5g) was taken in a small test tube and converted it with filter paper moistened with 1N sodium hydroxide. The test tube was placed for few minutes in boiling water. Filter paper was removed and examined under UV light for yellow fluorescence to indicate the presence of coumarins.

Table 1: Phytochemical screening result of ethanolic extract of aerial parts of *Cordia obliqua*

S.NO	TEST	ETHANOLIC EXTRACT
1	ALKALOIDS	
	a) Mayer's reagent test	+
	b) Dragendorff's test	+
	c) Hager's test	+
	d) Wagner's test	+
2	CARBOHYDRATES	
	a) Molisch's reagent test	+
	b) Fehling's test	+
	c) Benedict's test	+
3	GLYCOSIDES	
	a) Legal's test	
	b) Borntrager's test	
	c) Baljet test	
	d) Keller killiani test	
4	PHYTOSTEROLS	
	a) Libermann – Buchard's test	+
	b) Salkowski's test	+
5	FIXED OIL AND FATS	

	a) Spot test	
	b) Saponification test	
6	SAPONINS	
	a) Foam test	
7	PHENOLIC COMPOUNDS AND TANNINS	
	a) Ferric chloride test	+
	b) Gelatin test	+
	c) Lead acetate test	+
8	PROTEINS AND AMINOACIDS	
	a) Million's reagent test	+
	b) Biuret's test	+
	c) Ninhydrin reagent test	+
	d) Xanthoproteic test	+
9	GUM AND MUCILAGE	+
10	TEST FOR FLAVONOIDS	
	a) Sodium hydroxide test	+
	b) Concentrated sulphuric acid test	+
	c) Shinoda's test	+
	d) Alkaline reagent test	+
	e) Magnesium and HCl reduction test	+
11	TEST FOR LIGNIN	
12	TEST FOR TRITERPENOID	+
13	TEST FOR COUMARINS	

+ Positive -Negative

CONCLUSION

Many nutritional supplements contain phytochemicals that have been extracted from plants because plants are the only source of phytochemicals. Plant based food is the best way to get valuable phytochemicals and phytonutrients can contribute greatly to overall health. There are many phytochemicals present in plant sources and each works differently. The phytochemical screening of ethanolic extract of aerial parts of *Cordia obliqua* Willd. results reveal the presence of tannins may have a potential anticancer agents, phytosterols have cardiogenic and antimicrobial activity, alkaloids is used to treat heart failure, cancer and blood pressure, flavonoids reduce the risk of cardiovascular disease and used to treat viral, bacterial, fungal infections. Phenolic compounds are powerful antioxidants, Triterpenes strengthens immune function and showed potential hepatoprotective effect, saponins are used to treat hypocholesterolemia and interfere with DNA replication, thereby preventing the multiplication of cancer cells, gum and mucilages are frequently used as a binding, emulsifying, thickening and stabilising agents in pharmaceutical industries and also used as matrices for sustained release of drugs.

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Cite this article as:

S.Sivakrishnan and G.Veeramani. Phytochemical activity of aerial parts of *Cordia obliqua* Willd. Int. Res. J. Pharm. 2019;10(7):94-98 <http://dx.doi.org/10.7897/2230-8407.1007226>

Source of support: Nil, Conflict of interest: None Declared

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