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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF CELOSIA ARGENTEA (AMARANTHACEAE) AN ETHNOMEDICINAL PLANT

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ABSTRACT

Celosia argentea is a herbaceous plant grow locally in various region in the member of Amaranthaceae family. Plant bears simple and spirally arranged leaves, often pinkish or white flowers while fruits are globular and seeds are black. Traditional medicine herb used for treatment of fatigue, atherosclerosis, leucorrhoea and osteoporosis. Its seeds have been used for removing "liver heat" improving eyesight, clearing wind heat and as an anti- inflammatory agent the chemical constituents of this plant include mainly flavonoids, also used as ornamental plant.

The plant is an important medicinal leaf antidiarrhoeal value and it's different part used in Ayurvedic medicine. Sequential extraction carried out by using solvent viz. petroleum ether, ethanol and aqueous from leaf, root and seed of the plant were investigated for preliminary phytochemical analysis and antimicrobial activity. Phytochemicals are secondary metabolites produced by all plants in which some has medicinal uses. An in vitro antimicrobial activity and phytochemical analysis of various extracts of *Celosia argentea* viz. petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out. A total of 5 microorganisms (4 bacteria and 2 fungal strains) were used for antimicrobial activity. The extracts were screened for the presence of Phytochemicals and their effect on various microbes especially fungi such as *Candida albicans* (only single strain) and bacteria such as *E.coli, Staphylococcus aurus, Klebsiella pneumoniae*, *Salmonella tophi*, etc. Aqueous extract showed moderate inhibitory activity against *Staphylococcus aureus* (14mm). All the 3 extract showed a minimal antifungal activity when compared to antibacterial activity. Phytochemical analysis showed the presence of Alkaloids, Phytosterol, Fixed oils, Saponins and Phenolic compounds.

INTRODUCTION

Celosia argentea Linn belongs to family Amaranthaceae. In India, it is found to be grown as a weed of bajara fields. It is an hearbaceous, erect and branching plant. The plant body is erect up to 1.5-2 ft height. There are 17 species of *Celosia* recorded in India out of these 11 species of *Celosia* are found to be growing in Maharashtra. The family of Amaranthaceae consists of several important medicinal plant with wide range of biological activities and interesting phyto-chemical constituents. Medicinal plant are an important source for the therapeutic remedies of various ailments. Phytochemical are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. The major constituents are consists of carbohydrates, amino acid, protein and chorophyll while secondary metabolites of alkaloids, saponins, steroids, flavonoids, tanins and so on. Nature is the source of medicinal agent for thousands years and an impressive number of modern drug have been isolated from natural sources, many of these isolated where based on the uses of the agents in the traditional medicines. Medicinal herb is considered to be a chemical factory as it containsmultitude of chemical compounds like alkaloids, glycosides, saponins, resins, During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs

Several bioactive constituents have been isolated and studied forpharmacological activity. This plant based traditional medicine system continues to play an essential role in health care about 80% of world inhabitants relaying mainly on traditional medicines for the primary health care *Celosia*

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argentea belongs to Amaranthaceae. The Amaranthaceae family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, coumarins, saponins, and caffeic acid in the seed, leaves and roots. .

Celosia argentea is a herbaceous plant grown locally in various regions across the globe, in Lagos of Nigeria it is known as Lagos spinach, and among the yorubas it is known as *shoko yokoto*. it's had been discovered to have a lot of medicinal value apart from the nutritive value it is generally known for. It was reported that the Ethanolic extract yielded flavonoids, saponins, glycosides and tannins (kindayohan/celosia), this is the basis of its medicinal value and the ability to cure diverse diseases. Here are some of the listed medicinal portions made from *Celosia argentea*. Stems and leaves of celosia, bruised and applied as poultice, is used for treating of infected sores, wounds and skin eruptions. Poultice of leaves, smeared with honey, used as cooliing application to inflammed areas and painful affections such as buboes and abscesses. A seed are used to relieve gastrointestinal disorders and are antipyretic, improves vision, relieves fever associated with liver ailments. Seeds when in decoction or finely powdered, are considered antidiarrheal and aphrodisiac. The juice of the seeds forced into the nostrils is a cure for epistaxis. Whole plant used as antidote for snake-poison. Root used for colic, gonorrhea and eczema.

Decoction of the seeds with sugar is prescribed against dysentery. Flowers and seeds used for bloody stools, hemorrhoidal bleeding, leucorrhea and diarrhea. In Indian folk medicine, used for diabetes. Seeds traditionally used for treatment of jaundice, gonorrhea, wounds and fever. In Sri Lanka, leaves used for inflammations, fever and itching. Seeds used for fever and mouth sores. In China, flowers and seeds used in treatment of gastroenteritis and leucorrhea. (Kindayohan: philippine medicinal plant). Include celosia and other vegetables in your diet, it makes you healthier.

MATERIALS AND METHODS

Plant Collection and identification

Fresh plant material of *C. argentea* was collected from the Nasik district of Maharashtra, India. After collection of plant material firstly wash with clean water to remove sand and sometime dry it. Leave, Root and seed are shade dried or also dried by air dried method. The dried plant material crushes in grinder up to get a very fine powder.

Preparation of Extract

The 50 gm sample weight and is placed in porous thimble, made up to tough filter paper and is later placed in inner tube of soxhlet apparatus. The apparatus is fitted to 500 ml round bottom flask containing solvent and water condenser is used to reflex is round bottom flask is heated gently on heating mental.

Qualitative Phytochemical Analysis of Plant Extract

The phytochemical are essential to metabolism and chemical processes of plant body. The phytochemical are studied are alkaloids terpenoids and steroids, flavonoids, glycosides, tannins and saponins. Flavonoids are compounds found in fruits, vegetables and certain beverages that have diverse beneficial biochemical and antioxidant effects. The antioxidant activity of flavonoids depends on their molecular structure.

Identification Test

The test were do find the presence of active chemical such as alkaloids, glycosides, terpenoids, steroid, flavonoids, saponins, tannin by the following procedure.

Alkaloids

Detection of Alkaloid

Solvent free extract, 50 mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtered is tested carefully with various alkaloid reagent as follows :
Mayer's test (Evans, 1997).

To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of test tube. A white or creamy precipitate indicates the test as positive.
Wagner's (Wagner, 1993).

To few ml of filtrate, few drops of Wagner's reagent as added by the side of test tube. A reddish- brown precipitate confirms the test as positive.

Detection of Carbohydrates and Glycosides

The extract (100 g) is dissolved in 5 ml water and filtered. The filtered is subjected to the following test :-

Carbohydrates

Barfoed's test

To 1ml of filtered, 1ml of barfoed's reagent is added and heated on a boiling water bath for 2 min. Red precipitate indicates presence of sugar.

Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2min. A characteristic coloured precipitate indicates the presence of sugar.

Glycosides

50 mg of extract is hydrolysed with conc. Hcl 2hr on a water bath, filteredand hydrolysate is subjected to the following tests:-

Borntrager,s test *(Evans, 1997)*

To 2 mi filtered hydrolysate3ml of chloroform is added and shaken, chloroform layer is seperated and 10%ammonuim solution added to it pink colour indicates presence of glycosides.

Legal's test:-(Egwaikhide and Gimba 2007.)

50 mg extract is dissole in pyridine sodium nitroprusside solution is added and made alkaline using 10% sodium hydroxide to pink colour indicates presence of glycosides.

Detection of cardiac glycosides (Yadav and Agrwal, 2011)

Crude extract 2ml was mixed with 2ml glacial acetic acid containing 1-2 drops of 2% solution of fecl3. The mixture was poured into another test tube containing 2ml of conc. Of H2so4.A brown ring at the interphase indicates the presence of cardiac glycosides.

Detection of Amino acid and Protein (Fisher, 1968; Ruthmann, 1970)

The extract 100 mg is dissolved in 10 ml of D.W. and filtered through Whitman No.1 filter paper and the filtrate is subjected to tests for proteins and amino acid.

Million test (Rasch and Swift, 1960)

To 2ml filtrate, few drops of million's reagent are added. A white precipitate indicates the presence of proteins.

Ninhydrin test (Yassuma and Lchikawa, 1953)

Two Drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Detection of phenolic compounds and Tannins

Ferric chloride test (Mace, 1963)

The extract 50mg is dissolve in 5ml of D.W. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

Gelatin test (Evans, 1997)

The extract 50 mg dissolve in 5ml D.W. and 2ml of 1% solution of gelating containing 10% sodium chloride is added to it. White ppt indicate the presence of phenolic compounds.

Lade acetate test

The extract 50mg is dissolve in D.W. and 3ml of 10% lead acetate solution bulky white ppt indicates the presence of phenolic compounds.

Detection for Flavonoids (Siddiqui and Ali, 1997)

Four millilitres 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, 5-6 drops of concentrated hydrochloric acid was added and red colour for flavones.

Detection for fixed oils and fats

Spot test

A small quantity of extract is pressed between filter paper. Oil stein on the paper indicate the presence of fixed oil.

Detection for Volatile oil

In a volatile oils estimation apparatus, 50mg of powdered material (crude drug) is taken and subjected to hydro. Distillation. The distillate is collected is gradual tube of the assembly, where in the aqueous portion automatically seprate out from the volatile oil.

Detection for Gum and Mucilages (Whistler and Bemiller, 1993)

The extract 100mg is dissolved in 10 ml of distilled water and to this, 25ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

Detection for Terpenoids and Steroid

Test for Steroid :- (Siddiqui and Ali, 1997)

Four milligrams of extract was treated with 0.5ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour for Steroids.

Test for Terpenoids :- (Edeaga et.al.2005)

5ml extract mixed with 2ml chloroform and carefully added conc. H2so4 to form layer. A reddish – brown colouration at the interface shows positive result for the presence of terpenoids.

Phlobatanins

About 2ml aqs. Extract was added to 2ml of 1% Hcl and mixture was boiled. Deposition of red ppt was taken as evidence for presence of phlobatanins. **Antimicrobial Activity of Plant Extract**

To study antimicrobial activity following four bacterial and one fungal strain were used

Bacteria Fungi

- *Escherichia coli* 1. *Candida albicans*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Salmonella typhi*

The bacterial isolates were cultured on nutrient agar and incubated at 37 c for 24 hrs and the microorganisms were repeatedly sub-cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4 c .

Overnight broth culture of the respective bacterial strains was adjusted to turbidity equivalent to 0.5 Mcfarland standards .(0.2 ml culture of the organisms was dispensed into 20ml sterile nutrient broth and incubated for 24hrs and standardized at 1.5×10 CFU/ml by adjusting the optical density to 0.1at 600nm PERKIN ELMER UV-Spectrophotometer) 16.

Media Preparation

As per composition following media were prepared

PDA MEDIUM (For Fungi)

(potato dextrose agar medium)

1. Potato - 200gm
2. Dextrose- 20gm
3. Agar- 15gm
4. Distilled water- 1000ml

Nutrient Agar Medium (For Bactria)

1. Yeast extract- 10gm
2. NaCl - 5gm
3. Peptone - 10gm
4. Distilled water - 1000ml
5. Agar - 20gm

Phytochemical Screening

Phytochemical analysis of plant is presented in Table. The medicinal properties plant could be attributed to the presence of

bioactive compound in leaf, root and seed extract under study. All the extract have shown the presence of phytochemical. Analysis of aqueous leaf extract revealed the presence of phytochemicals such as Carbohydrates, Glycosides, Protein and amino acid, Saponin and Flavonoids while the aqueous seed extract presence of alkaloids, tannin, saponin, amino acid and protein, flavonoids, steroids, glycosides and carbohydrats.

The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites.

Table 3 Preliminary phytochemical analysis of Seed extract of *Celosia argentea*

Sr. No.	Phytochemical Test	Petroleum Ether	Aqueous
	Alkloids		
1	a- Mayer's Reagent	-	-
	b- Wagner's Reagent	-	-
	Carbohydrate		
2	a- Barfoed's Test	-	+
	b- Benedict's Test	-	+
3	Glycosides		
	a- Borntrager's Test	-	+
4	Cardiac glycosides	-	-
	Proteins and Amino acid		
5	a- Million Test	-	-
	b- Ninhydrin Test	-	-
6	Saponin	-	+
	Phenolic Compound		
7	a- Ferric Chloride Test	-	+
	b- Gelatin Test	-	-
	c- Lead Acetate Test	-	-
8	Flavonoids		
	a- Alkaline Test	-	+
9	Fixed Oil and Fats		
	a- Spot Test	+	+
10	Volatile Oil	+	+
11	Gum and Mucilage	+	+
12	Terpenoids	-	-
13	Steroids	-	-
14	Phobatanins	-	-

Table 2 Preliminary phytochemical analysis of Root extract of *Celosia argentea*

Sr. No.	Phytochemical Test	Petroleum Ether	Aqueous
	Alkloids		
1	c- Mayer's Reagent	-	-
	d- Wagner's Reagent	-	-
	Carbohydrate		
2	c- Barfoed's Test	-	-
	d- Benedict's Test	-	-
3	Glycosides		
	b- Borntrager's Test	-	-
4	Cardiac glycosides	-	-
	Proteins and Amino acid		
5	c- Million Test	-	-
	d- Ninhydrin Test	-	-
6	Saponin	+	+
	Phenolic Compound		
7	d- Ferric Chloride Test	-	-
	e- Gelatin Test	-	-
	f- Lead Acetate Test	-	+
8	Flavonoids		
	b- Alkaline Test	+	+
9	Fixed Oil and Fats		
	b- Spot Test	+	+
10	Volatile Oil	-	+
11	Gum and Mucilage	+	+
12	Terpenoids	-	-
13	Steroids	-	-
14	Phobatanins	-	-

They possess biological properties such as anti-apoptosis, anti-aging,anti-carcinogen, cardiovascular protection and improvement of endothelial function and cell proliferation activities. Terpenoids molecules they may have useful anticancer properties. Saponins has the property of precipitating and coagulating red blood cell. Steroids have been reported to antibacterial properties and they are very important compound especially due to their relationship with compounds such as sex hormone. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity several worker.

Table 3 Preliminary phytochemical analysis of Seed extract of *Celosia argentea*

Sr. No.	Phytochemical Test	Petroleum Ether	Aqueous
	Alkloids		
1	e- Mayer's Reagent	+	-
	f- Wagner's Reagent	+	+
	Carbohydrate		
2	e- Barfoed's Test	-	-
	f- Benedict's Test	-	-
3	Glycosides		
	c- Borntrager's Test	-	-
4	Cardiac glycosides	-	-
	Proteins and Amino acid		
5	e- Million Test	-	-
	f- Ninhydrin Test	-	-
6	Saponin	+	+
	Phenolic Compound		
7	g- Ferric Chloride Test	-	-
	h- Gelatin Test	-	-
	i- Lead Acetate Test	-	-
8	Flavonoids		
	c- Alkaline Test	+	+
9	Fixed Oil and Fats		
	c- Spot Test	+	+
10	Volatile Oil	+	+
11	Gum and Mucilage	+	+
12	Terpenoids	-	-
13	Steroids	-	-
14	Phobatanins	-	-

Antifungal activity

Table No.1 Antifungal activity of petroleum ether extract of *Celosia argentea*

Sr. No.	Extract	Zone of inhibition (mm) Candida albicans
1	*Leaves*	-
2	*Root*	-
3	*Seed*	-

Table No.2 Antifungal activity of aqueous extract of *Celosia argentea*

Sr. No.	Extract	Zone of inhibition (mm) Candida albicans
1	*Leaves*	-
2	*Root*	-
3	*Seed*	-

Antibacterial activity

Table No. 1 Antibacterial activity of petroleum ether extract of *Celosia argentea*

Sr. No.	Extract	Zone of inhibition (mm)			
		E. Coli	*S. aureus*	*K. pneumoniae*	*S. typhi*
1	Leaves	-	-	-	-
2	Root	-	-	-	-
3	Seed	-		-	-

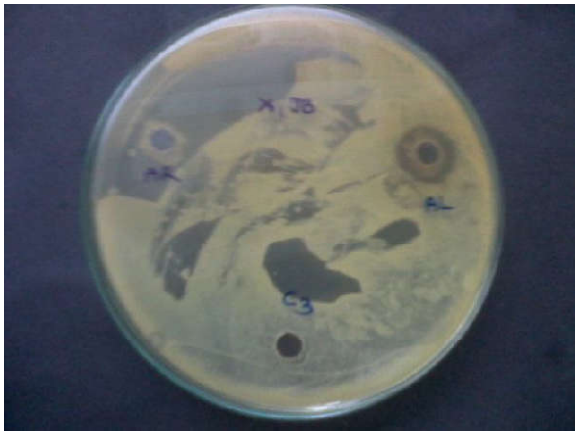
Table No. 1 Antibacterial activity of aqueous extract of *Celosia argentea*

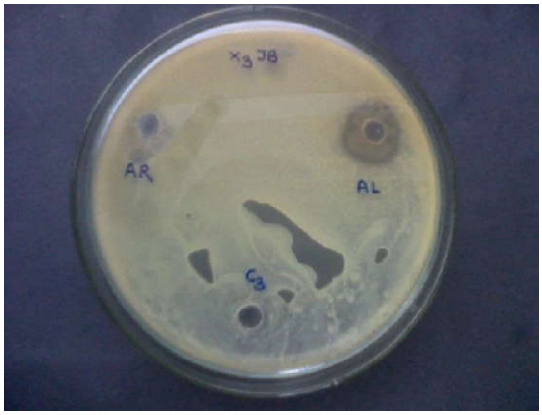
Sr. No.	Extract	Zone of inhibition (mm)			
		E. Coli	*S. aureus*	*K. pneumoniae*	*S. typhi*
1	Leaves	-	-	-	-
2	Root	-	-	-	-
3	Seed	-		-	-

CONCLUSION

The present study reveals that these plant under study can be use for the treatment of jaundice, gonorrhea, wounds, diarrhea, dysentery, fever, itching, anti-cancer. The seed posseses aphrodisiae, anti-pyretic, diuretic and anti-bacterial activity.

The demonstration of broad spectrum of anti-bacterial activity by *Celosia argentea* may help to discovered newchemical classes of anti-biotic substance. With the evidence of antibacterial and antifungal activities of the extracts of preparation under study to identify the chemical natures of the active principles as well as their mode of action on bacterial cells and thier roles in disease curing





Activity of aqueous leaf extract of *Celosia argentea* against *S. aureus* it shows Zone of inhibition

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