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POTENTIAL OF *CHENOPODIUM ALBUM* LINN FOR PHYTOCHEMICAL ACTIVITIES AND ANTIBACTERIAL SCREENING

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ABSTRACT

Throughout Africa, Asia, Europe, and North America, Chenopodium album Linn (Family: Chenopodiaceae) is a weed that is aggressively proliferating. This plant has been used as an antiparasitic, diuretic, hepatoprotective, laxative and sedative in folk medicine. Its leaves can be used as a wash or poultice on insect bites, rheumatoid joints, sunburn and swollen feet because they have antirheumatic, anthelmentic, antiphlogistic, moderately laxative and odontalogic characteristics. In addition to providing basic nutritional advantages, this plant is grown as a vegetable in India and has tremendous biological potential. The anthelmintic, antipruritic, contraceptive and sperm immobilising effects of this plant have been documented in earlier scientific studies. As a result, this plant has huge potential for thorough biological screening. So, the present study was carried out to assess its phytochemical and antibacterial properties in order to clarify the potential traditional usage of this plant. A methanol extract of Chenopodium album leaves was examined for its ability to inhibit the growth of *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes, Escherichia coli, Entrobacter, Klebsiella* and other bacteria (both gram-positive and gram-negative bacteria). The extract of Chenopodium album also contained proteins, glycosides, alkaloids, flavonoids, terpenoids, tannins, fixed oil, and lipids. The antibacterial activity were evaluated using the agar disc diffusion method. The outcomes shown that the antibacterial properties of *Chenopodium album Linn* suppress the growth of microorganisms in a dose-dependent manner. The outcomes demonstrate the antibacterial activity of *Chenopodium album* extract in the tested environment. Secondary metabolites are thought to be crucially produced by this plant. The results also imply that beneficial outcomes may emerge from scientific studies that regularly make use of herbs with established efficacy claims.

KEYWORDS

Phytochemical, Antimicrobial, *Chenopodium album*, Phytochemical, Bacteria, *E.coli* and Antibiotics.

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INTRODUCTION

Herbs are still used by 80% of world population, because of their easy availability and very negligible side effects. From ancient period medicinal plants are used for pharmacological studies. Knowledge of therapeutic value of these plants acts as sources for lead compound

development (Samanta, 2015)[1]. *Chenopodium album Linn*. (*C. album*) belonging to family *Chenopodiaceae*, is commonly called as *Bathua* (Hindi), *Chandanbetu* (Bengali), *Parupukkirai* (Tamil), *Pappukura* (Telgu) and *Katuayamoddakam* (Malyalam) in India. It is a fast growing annual plant which wildly grows as weed in Africa, Asia, Europe and North America. Tender branches and leaves are used as a vegetable in various parts of world. In India, it is locally used in curd known as "*Raita*". Leaves are often dried and stored for later use. Infusions of leaves were also used as a laxative. Hindu physicians recommended C. album for treatment of splenic enlargement and liver diseases (Priya, *et al*, 2010)[2]. In folk medicine, this plant has been used as antiparasitic, diuretic, hepatoprotective, laxative and sedative. Its leaves possesses antirheumatic, anthelmentic, antiphlogistic, mildly laxative and odontalgic properties, applied as wash or poultice to bug bites, rheumatic joints, sunstroke and swollen feet (Kokanova *et al*, 2009)[3]. This plant is used as vegetable in India and has high biological potential in addition to basic nutritional benefits. Previous scientific studies have reported the anthelmintic, antipruritic, antinociceptive, contraceptive and sperm immobilizing properties of this plant (Poonia and Upadhayay, 2015)[4].

Bathua, also known as Chenopodium album L. (family: Chenopodiaceae), is a herbaceous plant that is also eaten as a vegetable. It comes from Western Asia. It is a summer annual weed of the goosefoot species that grows erect quickly and can reach heights of up to one metre. The entire plant is covered in varying degrees of a waxy material, giving it the impression of being pale green. It frequently has culinary and medical uses. The plant and its components can treat cough, anorexia, piles, dysentery, diarrhoea and they can also destroy little worms (Bakshi, 1999)[5]. It is an invasive weed that thrives in nitrogen-rich soils and is particularly prevalent in temperate regions, especially on wasteland. The stems are glabrous, slightly succulent, and angled green. With a whitish coating on the underside, the opposing leaves can have a variety of appearances. Its pollen can cause allergies similar to hay fever (Wiart, 2006)[6]. A plant's medicinal value results from the presence of certain compounds such glycosides, alkaloids, volatile oils, resins, gums, and tannins, among others. The storage organs of plants, such as seeds, roots, leaves, bark, etc., typically contain the highest concentration of these active components (Ahmad *et al*, 2012)[7]. The plant's aerial sections contained 0.02% glycosides, according to the phytochemical study (Akhtar *et al*, 1999)[8]. As a result, this plant has huge potential for thorough biological screening. So, the current work was carried out to assess C. album leaf extracts for phytochemical and antimicrobial screening utilising some *in-vitro* test models in order to clarify the potential traditional usage of this plant.

MATERIAL AND METHODS

Plant Materials

The Chenopodium album leaf was procured from a nearby market in Raipur, Chhattisgarh, dried in the shade for a couple of days, then powdered, and kept in airtight containers for future study.

Extract Preparation

Twenty grammes of Chenopodium album leaf (20g) were extracted in a mixture of millipore water solvent and 50% methanol, then collected and concentrated in a water bath at 40-50^{o}C. An airtight container was used to keep the dry powder.

Phytochemical screening of the extract

The test was performed as per the method prescribe by Shahin Rizvi and Priyanka Pandey (2022)[9] using phytochemical screening, alkaloids, saponins, glycosides, proteins, phytosterols, flavonoids, terpenoids, tannins, fixed oil, and lipids were all examined.

Test for Alkaloids

1mL of mild hydrochloric acid was diluted with a minor amount of the extract before being filtered separately. The filtrate underwent treatment with Dragandroff's reagent. The emergence of organic precipitate indicates the presence of alkaloids.

Test for saponin

2g of powdered material was boiled in 20ml of distilled water in a water bath, then filtered. To create a stable, enduring foam, 10mL filtrate was mixed with 5mL distilled water and vigorously shaken. Three drops of olive oil were added to the froth, which was then vigorously stirred to produce an emulsion.

Test for Glycosides

A little amount of the extract was subjected to Fehling's test after being hydrolyzed for a few hours on a water bath with 5ml hydrochloric acid. 1ml of Fehling's A solution and 1ml of Fehling's B solution were added to 2ml of extract, which was then thoroughly mixed and heated. Yellow or red precipitate is a sign that there are reducing sugars present.

Test for Proteins

In order to test for Xantho protein, a small amount of the extract was dissolved in 5mL of water. 3mL of the extract were combined with 1mL of concentrated nitric acid. A white precipitate was the outcome. Prior to cooling under running water, the solution was heated for one minute. It was made alkaline by adding more than 40% NaOH. The emergence of an orange precipitate is a sign that protein is present.

Test for Phytosterol

The Salkowski method was used to find phytosterols. In this test, 1ml of concentrated sulfuric acid was added to 1g of plant extract and allowed to sit for 5 minutes. After shaking, the bottom layer takes on a golden yellow hue, indicating the presence of phytosterols.

Test for Flavonoids

A sulfuric acid solution was used to treat the extract. Anthocyanins are a yellowish orange colour, while flavones and flavonons range from orange to red in hue.

Test for terpenoids (Salkowski test

2ml of chloroform and 5g of each extract were mixed, and 3 ml of saturated H2S04 was then carefully added. An interface colour of reddish brown was produced to provide positive results for the presence of terpenoids.

Test for tannins

20ml of water were used to boil 0.5g of dried powder in a test tube, which was then filtered. A few drops of ferric chloride solution containing 0.1 percent was added and the colour was assessed for brownish green or blue-black hues**.**

Microorganisms

Gram-positive microbes among the microorganisms examined were *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and the Gram negative bacteria *Escherichia coli, Entrobacter, Klebsiella* and *Escherichia coli*. The National Chemical Laboratory (NCL) in Pune, India provided the bacterial strains used in this investigation. The bacteria were cultivated at 37 degrees Celsius in nutrient broth and maintained at 4 degrees Celsius on nutrient agar slants.

Antibacterial Assay

The agar disc diffusion method was used to investigate the antibacterial activity of *Chenopodium album* leaf extract at four different concentrations: 100, 75, 50 and 25mg/ml (Priyanka Pandey, *et al*, 2022)[10]. Following the preparation of Muller Hinton agar in accordance with the manufacturer's instructions, the plates were seeded with the appropriate microorganisms (*Gram positive bacteria; Bacillus subtilis, Bacillus cereus, Bacillus frimicutes and Gram negative bacteria; Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*). Discs with a diameter of 6 mm were produced using Whatmann filter paper No.24 and sterilised. The discs were then impregnated with the extracts and the solvent DMSO. Antibiotics for Gram positive and Gram negative bacteria include NX-Norfloxacin, OF- Ofloxacin, E-Erythromycin, and CFM- Cefixime. The control group used was bacteria. The zones of inhibition were measured using a measuring scale after the plates were incubated for 24 hours at 37 degrees Celsius. To assure accuracy, the afore mentioned experiment was carried out three times.

RESULTS AND DISCUSSION

Table No.1 contains the findings of the phytochemical examination of *Chenopodium album*

extract. Alkaloids, saponins, proteins, phytosterols, phenolic compounds, flavonoid terpinoids and tannins were examined in our test system and the results were found to be positive. However, tests for glycosides in both extracts yielded negative results.

Antibacterial Activity

Since the beginning of time, plants have been used for their antibacterial properties, which are demonstrated by the many secondary metabolites (phytochemicals) produced and deposited in either particular sections or all parts of the plant. The current imperative is to screen these substances and identify the bioactive components and their antibacterial capabilities. Methanol was discovered to be the most efficient solvent for maximising phytochemical separation, and preliminary examination of the extracts showed that *Chenopodium album* extract and secondary metabolites were both present in the leaves. Each phytochemical was isolated from the plant after being qualitatively identified. Its antibacterial activity was then tested against all Gram-Positive and Gram-Negative infections. The study found that the methanolic extracts of Chenopodium album at concentrations of 40mg/ml, 60mg/ml, and 100mg/ml, as well as control (methanol), significantly inhibited the growth of S. *aureus, B. subtilis, E. coli,* and *K. pneumoniae*.

Readings were taken in accordance with what was deemed to be zero hour-the invitation for microbial development. According to our findings about the antibacterial activity of the plant Chenopodium album, the antibacterial activity of 50% methanolic Basella alba against *S. aureus* is at its peak in 50% concentration after 12 hours of 7.33mm zone of inhibition. Nonetheless, 100% concentration has 11.33mm. The best concentration of extract in Bacilli Subtilis is 25%, which results in 1.33mm and 100%, which results in 2.33mm. 2.33mm is the 50% concentration in *Enterococcus*. 75% concentration in Proteus mirabilis is 4.33mm and 100% zone of inhibition is 5.66mm. In *E.coli,* the concentration of extract is good at 75% is 5.33mm and 100% is 6.66mm. A 1.33mm zone is seen at 25% concentration in Pseudomonas. In Klebsiella, a 1.66mm zone of inhibition is visible at 50% concentration. According to the observation above, the antibiotic activity of different concentrations (25, 50, 75, and 100%) against Staphylococcus *aureus, Bacillus subtilis, E.coli* and *Proteus mirabilis* was good. The extract's variable effectiveness against all bacteria is demonstrated in Table No.2 as a result.

Discussion

Since ancient times, these herbs have been used for medicine. This medicinal plant serves an active function in the development of novel pharmaceuticals as well as in the provision of conventional remedies to far-off markets. WHO estimates that 65% of all Indians take ayurvedic drugs, which are traditional treatments (Horio, 1993)[11]. Ayurvedic practises and therapies have been incorporated in nations outside of India for use in medical and, in some cases, general health purposes.

In ancient writings like Ayurveda, Atharva Veda, Charak Samhita, Sushruta Samhita and others, many species of Chenopodium have been said to offer a variety of medicinal characteristics (Bakshi *et al*, 1999)[5]. *C. album* has historically been used as an anthelmintic, cardiac tonic, digestive aid, laxative, and diuretic. In addition, it helps with peptic ulcers, dyspepsia, flatulence, strangulation, pharyngopathies, splenopathies, opthalmopathies and general malaise.

Table No.1 contains the findings of the phytochemical examination of Chenopodium album extract. Alkaloids, saponins, proteins, phytosterols, phenolic compounds, flavonoid terpinoids, and tannins were examined in our test system and the results were found to be positive. However, tests for glycosides in both extracts yielded negative results.

After 12 hours of a 7.33mm zone of inhibition, antibacterial activity of 50% methanolic extract of *Chenopodium album* against *S. aureus* is best at 50% concentration. Nonetheless, 100% concentration has 11.33mm. The best concentration of extract in *Bacilli Subtilis* is 25%, which results in 1.33mm, and 100%, which results in 2.33mm. 50% concentration in *Enterococcus* is 2.33mm. 75%

concentration in Proteus mirabilis is 4.33mm, and 100% zone of inhibition is 5.66mm. In *E.coli*, the concentration of extract is good at 75% is 5.33mm and 100% is 6.66mm. 25% concentration has a 1.33mm zone in Pseudomonas. In Klebsiella, a 1.66mm zone of inhibition is visible at 50% concentration. According to the observation above, the antibiotic activity of different concentrations (25, 50, 75 and 100%) against *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Proteus mirabilis* was good. Table No.4 illustrates the extract's variable efficacy against all bacteria. Kulcu *et al*[12] also noted the highest antibacterial activity in C. album (Kulcu *et al*, 2019). Great antibacterial activity was also reported by Ramproshad *et al*, (2018)[13] against a variety of harmful microorganisms.

The study calls for the introduction of fresh, physiologically safe and potent medications. Naturally occurring antibacterial and antioxidant compounds are present in plants. The *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* and other bacterial strains as well as many phytochemical analyses were successfully fought off by the methanolic leaf extract of *Chenopodium album*. It suggests that plant leaves contain phytochemical (medicinal) compounds for treating the many human ailments, but more research is required to substantiate this claim. According to the study, plants have strong antibacterial activity. However, more *in vivo* research is needed to understand how plants work as antimicrobial agents.

Table No.1: The study of phytochemical analysis of *Chenopodium album*

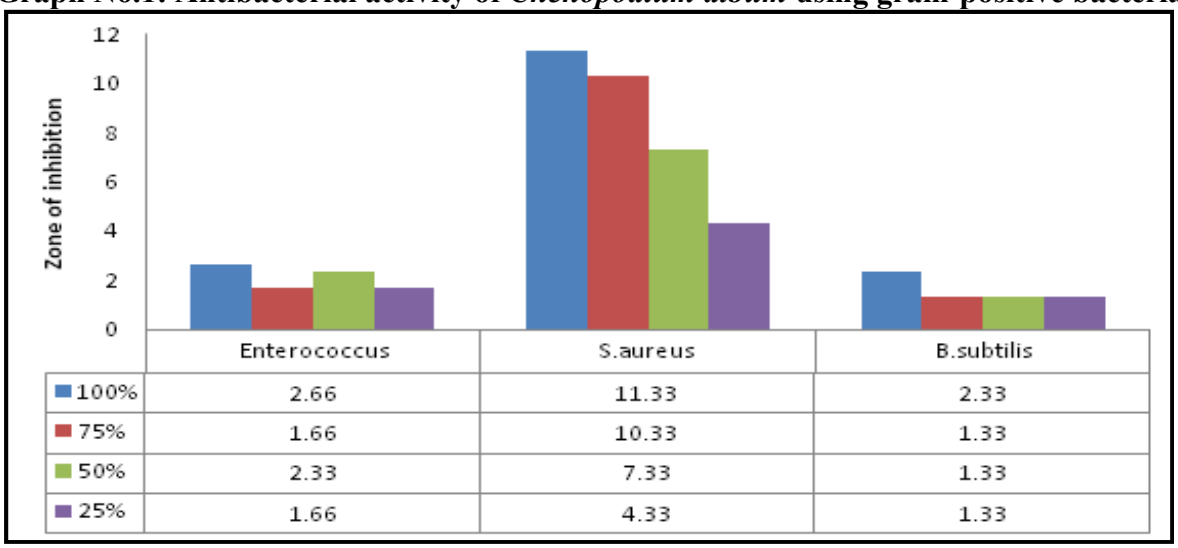
S.No	Phytochemical constituents	Extract of Leaf of *Chenopodium album*	Extract of Stem of *Chenopodium album*	Result
1	Alkaloids	+Ve	+Ve	Orange colour
2	Saponins	+Ve	-Ve	Emulsion
3	Proteins	+Ve	+Ve	Orange precipitate
4	Phytosterols	+Ve	+Ve	Golden yellow colour
5	Phenols	+Ve	-Ve	Violet colour
6	Flavonoids	+Ve	+Ve	Orange colour
7	Terpinoids	+Ve	+Ve	Radish Brown colour
8	Tennins	+Ve	-Ve	Brownish green colour
9	Glycosides	+Ve	+Ve	Violet colour

Where: + = Present, - = Absent

Table No.2: The study of anti-bacterial activity of *Chenopodium album* leaf extracts using Disk Diffusion method (Mean±SE)

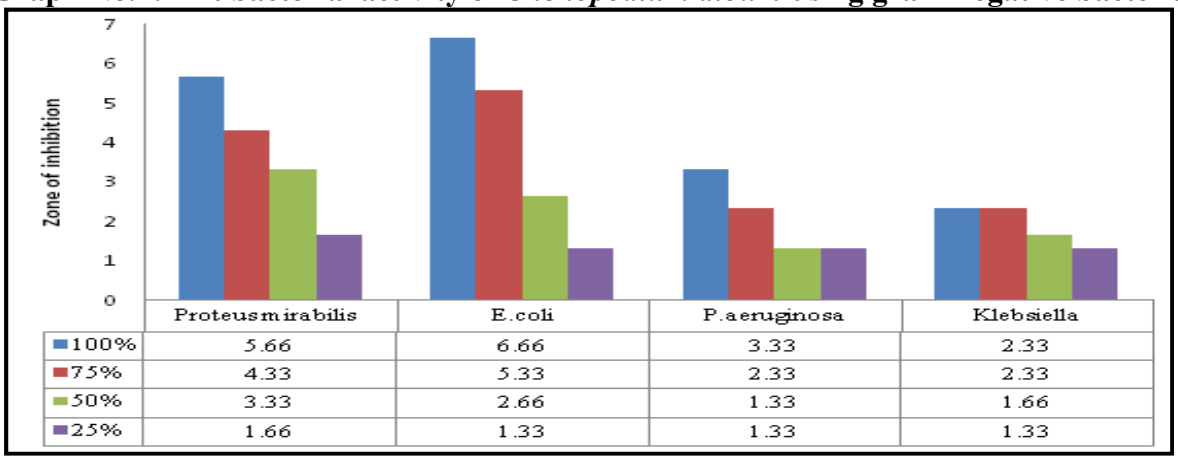
S.No	Bacterial Stain	Bacteria use	Zone of Inhibition (In MM)			
			100%	75%	50%	25%
1	Gram Positive(+)	*Enterococcus*	2.66 ±2.99	1.66±3.08	2.331±.50	1.663±.0.8
		S. aureus	11.33±1.51	10.33±1.51	7.331±.13	4.330±.0.83
		B. subtilis	2.33±3.08	1.33±1.05	1.33 ±1.05	1.33 1±.05
2	Gram-Negative (-)	*P. milrabillis*	5.661±.50	4.33±1.13	3.33 ±1.51	1.66 3±.08
		E.coli	6.66±3.84	5.33±3.08	2.65±3.08	1.33±3.08
		P. aeruginosa	3.33±3.08	2.33±3.08	1.33 ±3.08	1.33±3.08
		Klebsiella	2.33±3.08	2.33±3.08	1.66 ±3.08	1.33±3.08

Graph No.1: Antibacterial activity of *Chenopodium album* using gram-positive bacteria



	Enterococcus	S.aureus	B.subtilis
100%	2.66	11.33	2.33
75%	1.66	10.33	1.33
50%	2.33	7.33	1.33
25%	1.66	4.33	1.33

Graph No.2: Antibacterial activity of *Chenopodium album* using gram-negative bacteria



	Proteus mirabilis	E.coli	P.aeruginosa	Klebsiella
100%	5.66	6.66	3.33	2.33
75%	4.33	5.33	2.33	2.33
50%	3.33	2.66	1.33	1.66
25%	1.66	1.33	1.33	1.33

Chenopodium album *Chenopodium album dried leaves*

CONCLUSION

Natural components from traditional medicinal plants improve health and lessen afflictions. The global use of modern dyes has increased scientific analysis of the varied pharmacological effects of medicinal plants (Hemalatha 2013)[14]. The use of plant-derived products in natural medicines may lessen negative side effects, and plant chemicals have anti-mutagenic properties (Mastanm *et al*, 2012)[15]. In addition to having therapeutic qualities, *C. album* is a good source of useful nutrients.It can be incorporated to improve the nutritional value, health, and customer focus of various extruded food products. Its leaves can improve extruded goods' chemical and nutritional characteristics as well as their ability to serve as functional foods. The plants may have tremendous therapeutic usefulness because they also have high biological activities. In many parts of the world, commercial exploitation of *C. album* is still a long way off. The nutritional, phytochemical, antioxidant and antibacterial properties of "Bathua" were examined in order to employ it as a functional food, nutraceutical and flavouring ingredient with health advantages. There are numerous anecdotal stories that suggest bathua intake may improve a variety of ailments. It can also be utilised as an ingredient in meals to create processed goods like raita and paratha. Given that it doesn't contain any hazardous substances, it can be utilised as animal feed. These findings back the positive health claims as well. Hence, there is a vast opportunity for further study and pharmaceutical research on *C. album*.

It is possible to separate the active components and further assess them in order to create viable medications. Its antibacterial, antifungal and antioxidant properties support the biological significance of this plant even more. These studies ought to open the door for the use of *C. album* in areas where green leafy crops are grown but have not yet been exploited commercially. Increased societal awareness and subsequent increased use of this plant may go a long way towards preventing not only deficiency diseases and disorders associated with ageing and muscular degeneration, but also chronic degenerative diseases like cancer and cardiovascular disorders, which will ultimately be very beneficial to the rural community. In order to investigate the antibacterial, phytochemical, and antioxidant capabilities of this plant, a study was conducted because it is frequently used in Indian cuisine and because of its health benefits. The study's findings suggest that Chenopodium has strong antibacterial capabilities as well as excellent antioxidant effects. One or more of the chemical compounds identified in the extract may be the cause of this plant's action. The exercise could help alleviate a number of illnesses and health issues.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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