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Dr. Manju Madhavan
Asst Professor,
Department of Botany
Vimala College, Thrissur.

Correspondence:
Dr. Manju Madhavan
Asst Professor,
Department of Botany
Vimala College, Thrissur.

Quantitative Estimation of total phenols and antibacterial studies of leaves extracts of *Chromolaena odorata* (L.) King & H.E. Robins

Dr. Manju Madhavan

Abstract
Chromolaena odorata (L) King & H.E. Robins, a species of Asteraceae family. They are known as devil weed, Communist weed. The plant *C. odorata* is known for its medicinal importance among the tribal population. It is a common practice to use the leaf extract is to heal wounds.In the present study, an attempt is made to evaluate the total phenols in leaf extracts of *Chromolaena odorata* and to find out the antibacterial effect of the extracts against wound infecting bacteria *Staphylococcus aureus* and *Escherichiacoli*. Total phenolic content were determined using Catechol as standard. Phenol is determined in the distilled water, ethanol and acetone extracts. Maximum phenol content is found in acetone extracts. The antibacterial activities of the extracts were determined by the disc method Acetone extract showed maximum zone of inhibition and water extract showed the least.

Keywords: Chromolaena, antibacterial activity, phenol content, medicinal plant, wound healing.

1. Introduction
Medicinal plants are nature's priceless gift to humans. Herbs are used traditionally to cure many diseases both developing and developed countries. Even though development in the field of modern medicine temporarily subdued the traditional herbal medicine, now it has staged a comeback and a "herbal renaissance" is blooming all across the world. According to WHO in 2008, nearly 80% of the world's population are depending on herbs for their health care needs. The antibacterial or microbial activities of plants are attributed to the presence of secondary metabolites in plants. Phenolic acids, one important class of secondary metabolites widely spread throughout the plant kingdom. Studies have shown that natural phenols exhibit good antibacterial activity. Phenol also known as carbolic acid is an organic compound with chemical formula C_6H_5OH. Phenolic acids are easily absorbed through the walls of our intestinal tract, and they may be beneficial to our health because they work as antioxidants that prevent cellular damage due to free-radical oxidation reactions. They may also promote anti-inflammatory conditions in our body when we eat them regularly. Phenolic compounds are essential for the growth and reproduction and are produced as a response for defending injured plants against pathogens in some plants, they are secreted by the root system in the form of phytoalexins to check the growth of nearby plants [1]. Some phenolics are water soluble, some others are soluble in organic solvents, and still others are insoluble polymers. Although pharmacological industries have produced a number of new antibiotics in current clinical use, in last decades, resistance of microorganisms to these drugs has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents. So more and more plants, crude drugs are analysed for the antimicrobial properties.
Chromolaena odorata (L) King & H.E. Robins, a species of Asteraceae family and known in English as Siam weed, is a perennial shrub native of central & South America. From there they extends its territory to the Asian countries like India, China, Bangladesh, Thailand etc. They are known as devil weed, Communist weed etc. It expands rapidly at the onset of the rainy season and forms impenetrable tangles that may ultimately shade out indigenous vegetation. The plant's ability to thrive in a wide variety of soil in the tropics and its short juvenile stage, flowering in dry season, prolific seed production and strong ability to re-sprout after burning during land preparation all contribute to its invasiveness. It is a much branched perennial shrub that forms dense tangled bushes 1.5 to 3m in height in open condition, and occasionally reaching 6-10m by scrambling up other taller vegetation.

The stems are circular, hairy or almost smooth and much branched. The leaves are opposite triangular shaped, young ones slightly reddish purple, have toothed margins, with 3 main veins, and give off a pungent odour when crushed. Flower pale blue or white. Seeds are borne in the composite flower heads. The individual seed is about 5mm long with a pappus with angled hooks to aid seed dispersal. As the species name *C odorata* suggests, the leaves emit a pungent odour when crushed. It is a herb or sub shrub with many paired branches grow after main stem [2].

The plant *C. odorata* is known for its medicinal importance among the tribal population. It is a common practice to use the leaf extract is to heal wounds. *C. odorata* is reported to have antispasmodic, antiprotozoal, antitrypanosomal, antibacterial and antihypersensitive activities. It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatotropic activities. In the southern part of Nigeria, the leaves of *C. odorata* are used for wound dressing, skin infection and to stop bleeding according to Hill [3]. As the plant is a weed it is available in large quantities. Lot of medicinal properties are attributed to this plant. Important property is its power of wound healing. In the present study, an attempt is made to evaluate the total phenols in leaf extracts of *Chromolaena odorata* and to find out the antibacterial effect of the extracts against wound infecting bacteria *Staphylococcus aureus* and *Escherichia coli*.

2. Materials and Methods

2.1. Source of plant material

The plants grown on the road side and uninhabited plots near Vimala College, Thrissur were collected (Fig 2a).The Fresh leaves were collected from the plant. Dust and debris were removed from the plant parts and shade dried. Shade dried leaves were grinded to fine powder by a domestic grinder.

2.2. Preparation of extract

The extract was prepared using distilled water, acetone and ethanol. The extract was prepared by soaking 20gms of dry leaf powder with 100ml of various solvents for 48hours and kept in a magnetic stirrer for 6 hours. The extracts obtained from various solvents through filtration were kept for evaporation in hot air oven to remove the excessive solvents. The dried solvent extracts were stored in a cool dry place.

2.3. Estimation of total phenol

Total phenolic content were determined according to the method of Singleton and Rossi 1965, using Catechol as standard [4]. One ml(1mg/ml) of the extracted sample from the respective solvents were mixed with equal volume of Folin and Ciocalteu's phenol reagent and incubated for 3 minutes at room temperature, to this 1 ml of saturated sodium bicarbonate (3.5%) was added and final volume was made up to 10 ml with distilled water. The reaction mixtures were kept in dark for 90minutes and absorbance was read in Spectrophotometer at 650 nm. The standard solution of phenol was prepared with 200 mg of catechol dissolved in distilled water and made upto 100 ml. The working solution (10 µg, 20 µg, 30 µg, ---140µg) was prepared by diluting the stock solution with distilled water in the proportion 1: 10. Standard graph plotted for catechol (Fig.1) where '**Y**' is the concentration of total phenols in µg and 'X' is the optical density. The phenol concentration in the extract was calculated from the graph and expressed as µg of catechol equivalent per mg of the extract.

2.4. Disc diffusion Method

The antimicrobial activities of the extracts were also determined by the disc method [5]. 15ml of the nutrient agar medium was dispensed into pre sterilised petridishes to yield a uniform depth for bacterial inoculation. The sterile discs (Himedia) were impregnated with various extracts (1mg/ml) and were placed on the agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Streptomycin (10µg) was used as positive control. The discs were spaced far enough to avoid overlapping rings of inhibition. Finally, the petridishes were incubated for 24 hours at 37 °C for bacteria. The diameter of zone of inhibition is indicated by the clear area which was devoid of growth of microbes. The treatments were repeated thrice and the mean is taken.

The bacterial cultures of *S. aureus* and *E.coli* were procured from the department of Microbiology, St Mary's College Thrissur. The liquid broth for bacterial culture were prepared by dissolving 13gms of nutrient broth in 1000ml distilled water.5ml of this medium were dispensed in test tubes and autoclaved. This autoclaved medium in test tubes were taken to the microbiology department and inoculated with the bacterial strains. These test tubes were incubated in the incubator for 2 days in 37 °C.

3. Result

In the present work quantification of total phenol and the antibacterial activity of *C. odorata* leaves extracts were estimated. Antibacterial activity of the leaf extracts were assessed against the wound infecting bacteria namely *E.coli* and *S. aureus*.

About 20g of the powdered leaves were soaked in 100ml of solvents such as ethanol, acetone and water. Water soluble extractive value was found to be 1.23g and shows light brown colour. Ethanol extract showed an extractive value of 1.20g with deep greenish black colour, whereas acetone extract showed light green colour and the extractive value was found to be 0.50g (Table 1). The water extract showed high extractive value, which is slightly greater than that of ethanolic extract.

Table 1: Shows the solvent used for extract preparation, colour of extract and weight of extract obtained from *C.odorata* leaf powder

Solvent	Colour of the extract	Extractive Value (gms)
Acetone	Light green	0.50
Ethanol	Deep greenish black	1.20
Water	Light brown	1.23

Presence of total phenols in the extracts (1mg/ml) was characterized by the presence of bluish green colour (Figure 2b). Phenol content in the extract was quantified from standard graph plotted for catechol (Figure 1). Phenol content in water extract was found to be 52µg, acetone 106µg and ethanol 100µg. Maximum phenol content observed in acetone extract (Table 2). Water extract showed less phenol content.

Table 2: Quantity of phenol in different extracts of *C.odorata* expressed as µg catechol equivalent per mg of extract

Extract	OD Value	Conc. Of Phenol
Acetone	0.62	106 µg
Ethanol	0.60	100 µg
Water	0.28	52µg

The water, ethanol and acetone extracts were studied against wound infecting bacteria *S. aureus*. and *E.coli*. 1mg/ml of each extract is taken and were tested against these bacteria. The ethanol and acetone extracts showed antibacterial activity. This was clear with the zone of inhibition obtained (Fig.2d, e). Streptomycin disc was used as positive control (Fig.2 c). The antibacterial activity showed that the ethanolic extract of *C. odorata* exhibited a zone of inhibition of 10.6mm against *S. aureus* and *E.coli*. Acetone extract showed only 12.6mm of inhibition against *S. aureus* and *E.coli*. Whereas water extract didn't show any activity against *E.coli* (Table 3).

Table 3: Showing the Diameter of Zone of inhibition (mm) obtained by antibacterial activities of different leaf extracts of *C.odorata*.

Extracts	***E.coli***				***S. aureus***			
Treatment 1	R1	R2	R3	Mean	R1	R2	R3	Mean
Cetone	12 mm	14 mm	12 mm	12.6mm	14 mm	12mm	12mm	12.6mm
Ethanol	10mm	12mm	5mm	10.6mm	12mm	10mm	10mm	10.6mm
Water	-	-	-	-	1mm	1mm	2mm	3.2mm
Streptomycine Disc	20mm				22mm			

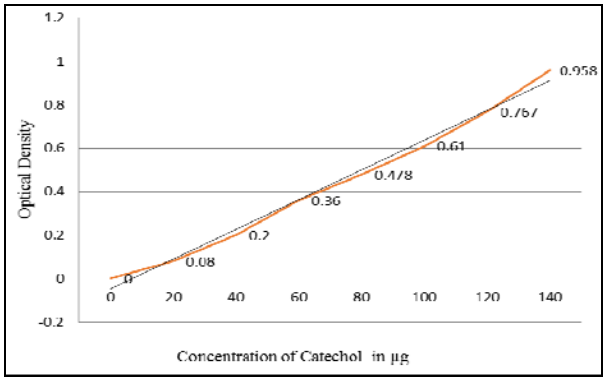

Fig 1: Catechol Calibration Curve

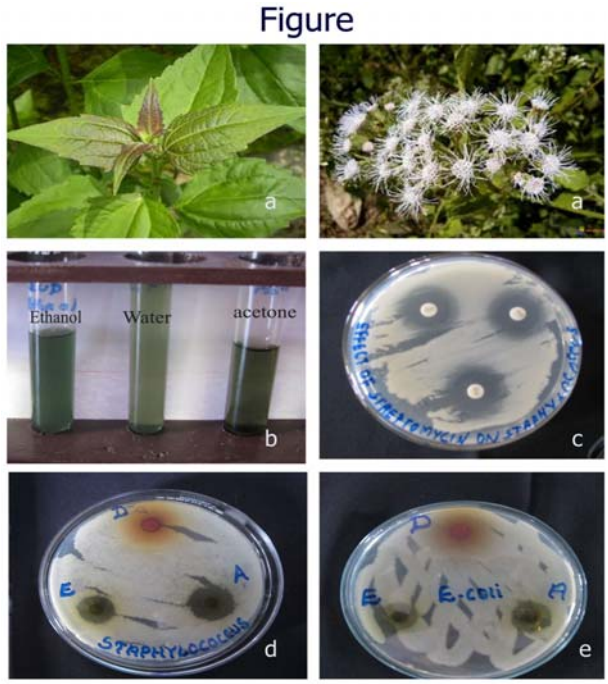

Fig2 . a. *Chromolaena odorata* plant **b.**Qualitative estimatiom of phenols in ethanol,water and acetone extract.**c** Effect of streptomucin on S aureus.**d.** Effect of extracts on S aureus. **e.** Effect of extracts on E c

4. Discussion

In the present study, *C. odorata*, commonly known as 'communist weed" or 'Siam weed', is studied for the phenolic content and antibacterial properties. This plant is considered to be a harmful weed due to its highly invasive, allelopathic nature. It grows in pastures, marginal lands, open areas, dry deciduous forests and interior shrub jungles, where it is highly competitive and does not let other flora grow. Although it was used traditionally for its healing properties, it never enjoyed the status of a medicinal herb [6]. Instead, efforts were always made to eradicate the so called weed. In the present scenario, microbes are getting more and more resistant, allopathy medicines are showing side effects so more and more crude drugs obtained from plants are analysed for antibacterial properties. So in the present work, this plenty available plant has been studied for its antibacterial activity.

Natural preparations from plants, crude extracts contain phenolic compounds and exhibit antibacterial activity [7]. Plant phenolics, especially dietary flavonoids, are currently of growing interest owing to their supposed functional properties in promoting human health. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavour and also in providing health beneficial effects. They also serve in plant defence mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores. The aqueous extract and the decoction from leaves of this plant have been used throughout Vietnam for the treatment of soft tissue wounds and burns for decades. A product made from Chromolaena named eupolin have already been licensed for use in Vietnam for soft tissue burns and wounds [6]. In the present study ethanol, acetone and water extracts were qualitatively and quantitatively evaluated for the presence of phenol. The presence of phenol was detected on all the three extracts by the bluish green colouration, quantitatively found maximum in acetone and minimum in water.

The antibacterial activity is a major factor in the wound healing property expressed by the herbs [8]. In this work, antibacterial study of the extracts was conducted aganist the wound infecting bacteria *S. aureus* and *E.coli*. Acetone extract showed maximum zone of inhibition and water extract showed the least. The studies by Irobi showed that antibacterial activity of ethanolic extracts of *C odorata* and a zone of inhibition obtained varied from 5mm to 24 mm against different microbial strains [8]. In one of the experiments conducted by Taleb-Contini *et al.* [9]. he crude extracts (dichloromethanic and ethanolic) from *Chromolaena* have been evaluated against 22 strains of microorganisms including bacteria (Gram-positive and Gram-negative) and yeasts. Vital and Windell in their works with *C odorata*, crude extracts have shown activity, mainly against Gram-positive bacteria. It was found to be particularly active against *Staphylococci* [10]. In our studies phenol content in the ethanol and acetone extract is found to be more or less equal and the zone of inhibiton obtained also is the same against both the bacteria.

This study too confirms the presence of phenol in leaf extracts obtained by using different solvents. These extracts also showed antibacterial activity. Efforts should be made to exploit the medicinal properties of this abundant herb. The example of this herb indicates the importance to consider and evaluate the abundantly occurring weed species on this planet as potential sources of medicines than as invasive flora.

5. References

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