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Phytochemical and Pharmacological Studies of *Chromolaena odorata* against Human Pathogens

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Abstract: *Chromolaena odorata* colloquially known in English by numerous names including Siam weed, trifid weed, bitter bush, Jack in the Bush, Christmas bush and baby tea. This plant has great number of medicinal values inclusive of wounds healing property, anticancer activity, Antimicrobial property etc. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. The fluorescence analysis of leaf powder of *C. odorata* was carried out after treating with several solvents represented with colour changes which showed its fluorescence activity. Phytochemical analysis of *C. odorata* leaves extracts revealed the presence of flavonoids and carbohydrate in all tested solvents. Tannins, Saponins and glycosides are present in aqueous, ethanol and acetone extracts but absent in hexane extracts. Oxalate present in ethanol, acetone and hexane extract but not in aqueous extract. Coumarin present in ethanol and acetone extracts and absent in aqueous and hexane. Phlobatanins and amino acids were present in aqueous extract alone and these compounds were absent in other tested solvents namely ethanol, acetone and hexane. The antioxidant activity of *C. odorata* solvents extracts were high to the ethanol followed by aqueous, acetone and hexane and the O.D value of the tested samples were used to calculate the Percentage of Inhibition. The result of reducing power ability of the plant leaves have a dose-dependent effect as the reducing power ability of the plant increased with increasing concentrations from 12.5 µg/ml to 200 µg/ml. The results obtained suggests that the ethanolic and acetone leaf extracts of *C. odorata* are potential natural source for drug development to treat bacterial infections. The results obtained from antibacterial activity suggests that the ethanolic and acetone leaf extracts of *C. odorata* are potential natural source for drug development to treat bacterial infections compared to other tested solvent extracts. The anti-helminthic activity revealed that the *C. odorata* ethanol extract was endowed with potent anti-helminthic property as compared to other extracts like aqueous, acetone and hexane. But these extracts also possess significant activity. The positive control Albendazole treatment was able to cause paralysis and death to the worm at 8.33 min and 5.33 min, respectively at 100 mg/ml concentration.

Keywords: *Chromolaena odorata*, Fluorescence, Phytochemical, Antioxidant, Antibacterial, Anti-helminthic

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Introduction

Chromolaena odorata (L.) R.M. King & H. Rob. belongs to the largest family (Asteraceae) of flowering plants, which contains about 900 genera and 13,000 species (Heywood *et al.*, 1977; Akinmoladun *et al.*, 2007). *C. odorata* is an ornamental plant considered to be one of the most invasive environmental weeds of roadsides, wastelands and other exposed areas in the world (Warea, 2004). The leaves of this plant have been found to be a rich source of flavonoids including quercetin, sinensetin and padmatin (Vijayaraghavan *et al.*, 2017). *C. odorata* is known by other names such as baby tea, bitter bush, Armstrong's weed, butterfly weed, devil weed, king weed, Christmas bush, Siam weed, eupatorium, paraffin weed and turpentine weed (Vijayaraghavan *et al.*, 2017). It contains bioactive compounds such as steroids, flavonoids, alkaloids, tannins and saponins, which can act singly or synergistically to exert different biological effects (Olawale *et al.*, 2022). *C. odorata* for treatment of dozens of diseases as well as for the protection of crop and stored staple food from pest attacks (Touré *et al.*, 2014). In traditional medicine, the leaf decoction is used as a cough remedy and as an ingredient with guava leaves and lemon grass for the treatment of malaria. Other traditional and medicinal uses include astringent, anti-diarrheal, antihypertensive, antispasmodic, anti-inflammatory, tonic, antipyretic, diuretic and heart tonic. The fresh leaves and extract of *C. odorata* are being used as the traditional herbal treatment in some countries for burns, skin infection and soft tissue wounds. The scientific classification of *C. odorata* and its natural history is illustrated in Tables 1 and 2, respectively. In the present study an attempt has been made to study Phytochemical and pharmacological analysis of *C. odorata* against various human pathogenic bacteria.

Materials and Methods

Collection and preparation of *C. odorata*:

The plant *C. odorata* was collected from Village near Alangayam and it has been identified and authenticated by Dr. N. P. M. Mohamed Tariq,

Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi, India. After identification, the plants *C. odorata* (Fig. 1) were processed for extraction. The *C. odorata* was thoroughly cleaned with distilled water to remove dust particles and shadow dried at room temperature and reduced to coarse powder using a mechanical grinder. The powder was subjected to extraction by maceration using various solvents like Acetone, Ethanol, Hexane and Water to obtain their respective extracts. To 10 g of the powder in 100 ml solvent Acetone, Ethanol, Hexane and Water were added and stirred occasionally in orbital shaker. The mixture was filtered on the 2nd day and the solvent was evaporated at room temperature for 18-24 h to obtain a solid mass, which was stored in refrigerator (4 °C) for further use (Fig. 1).

FLUORESCENCE ANALYSIS TEST:

A small quantity of finely powdered *C. odorata* leaves sample were placed on a grease free microscopic slide and 1-2 drops of freshly prepared solution was added, mixed by gently tilting the slide and waited for 1-2 min. Then the slide was placed inside the UV viewer chamber and viewed in day light and ultraviolet radiations. The colour observed by application of different reagents in various radiations were recorded (Chang *et al.*, 2002; Mukherjee. 2002).

PHYTOCHEMICAL ANALYSIS:

Plant extract obtained with Aqueous, Ethanol, Acetone and Hexane were evaluated for the presence of terpenoids, steroids, flavonoids, alkaloids, saponins, tannins, phlobatannins, phenols, anthraquinones etc. using the method of Harbone (1973).

Alkaloids:

Wagner's test:

To 1 ml of extract, 1 ml of Wagner's reagent was added and observed the colour changes.

Amino Acid:

Table 1: Scientific Classification of *Chromolaena odorata*

Kingdom :	Plantae
Clade :	Tracheophytes
Clade :	Angiosperms
Clade :	Eudicots
Clade :	Asterids
Order :	Asterales
Family :	Asteraceae
Genus :	Chromolaena
Species :	<i>C. odorata</i>

Binomial name: *Chromolaena odorata* (L.) R. M. King & H. Rob

Table 2: Natural History of *Chromolaena odorata*

Flower	In much branched, corymbose panicles; capitula white. Flowering from December-March.		
Fruit	An achene, scaly without, angles thickened. Fruiting throughout the year.		
Field tips	Leaves 3-nerved from base.		
Leaf Arrangement	Opposite-decussate		
Leaf Type	Simple		
Leaf Shape	Obovate to deltoid-ovate		
Leaf Apex	Acute		
Leaf Base	Acute to truncate		
Leaf Margin	Coarsely serrate		
General Habitat	Less in the plains, introduced. Aggressive colonizer. Hills, lower slopes, 500-1000m. Native of south America. Widely naturalized in tropical Asia.		
Distribution	Found along the roadsides and waste places from plains to 1000m. Common. Native of tropical America naturalized widely in tropical Asia.		
Medicinal Uses (Phan <i>et al.</i> , 2001; Warea, 2004, Taleb-Contini <i>et al.</i> , 2006; Akinmoladun <i>et al.</i> , 2007; Ling <i>et al.</i> , 2007; Vital and Windell, 2009; Kouamé <i>et al.</i> , 2013; Wang <i>et al.</i> , 2014)			
Wounds Healing	Burns	Skin infections	Fever
Dysentery	Swellings	Analgesic	Cuts and Wounds
anticancer	Antidiabetic	Anti-hepatotoxic	Anti-inflammatory
Antimicrobial	Antiplasmodic	Antiprotozoal	Antitrypanosomal
Antifungal	Antihypertensive	Inflammatory	Astringent
Diuretic	Hepatotropic	Periodontitis	Antipyretic
Sore throats	Colds	Leech bites	

Xanthoprotein test:

To 1 ml of extract, 1 ml of Concentrated HNO₃ was added (White precipitate was formed). It was heated for 2-3 min and cooled. Then 1 ml of 20% of NaOH was added.

Carbohydrate:***Benedict's test:***

To 1 ml of extract, 1 ml of Benedict's reagent was added and heated in a boiling water bath for 2-3

min. The reaction was observed and the results were recorded.

Phenol Test:***FeCl₃ test:***

To 1 ml of the extract, 1 ml of 5% ferric chloride was added. The action was observed and the result was noted.

Flavonoids Test:***Alkaline reagent test:***

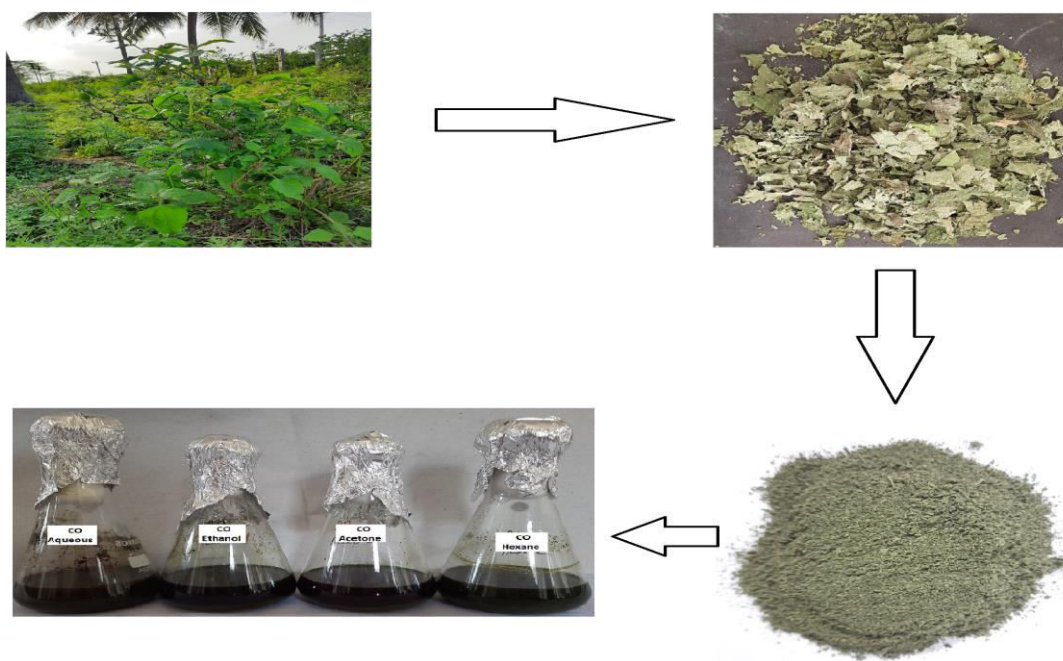


Fig. 1: Collection, preparation and extraction of *C. odorata*.

To 1 ml of extract, 1 ml of 10% NaOH was added and observed. Fluorescence was noted.

Tannins:

FeCl₃ test:

To 2 ml of the extract, 2 ml of 5% ferric chloride was added. The appearance of colour was observed and noted.

Saponin:

Foam test:

To 2 ml of the extract, 2 ml of the dis. H₂O was added and shaken vigorously. The formation of stable foam was observed and recorded.

Terpenoids:

Salkowskis test:

To 1 ml of extract, 2 ml of chloroform and 3 ml of H₂SO₄ were added. The appearance of colour was observed and noted.

Phlobatanins:

1% Hydrochloric Acid test:

To 2 ml of the extract, 2 ml of the 1% HCl was added and heated in boiling water bath and the presence of colour was observed and noted.

Quinones:

Hydrochloric acid test:

To 1 ml of the extract, 1 ml of the conc. HCl was added. The appearance of colour was observed and noted.

Coumarin:

Sodium hydroxide test:

To 1 ml of the extract, 1 ml of 10% sodium hydroxide was added. The appearance of colour was observed and noted.

Oxalate:

Glacial acetic acid test:

To 3 ml of extract, 1 ml of glacial acetic acid was added. The appearance of colour was observed and noted.

Glycoside:

Keller-Killiani test:

To 2 ml of the extract, 2 ml of the glacial acetic acid and few drops of the 5% FeCl₃ and conc.H₂SO₄ were added. The appearance of colour was observed and noted.

ANTIOXIDANT ACTIVITY:

The Antioxidant activity of the solvents extracts of *C. odorata* were performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A solution of 0.135 mM DPPH was freshly prepared by dissolving 4 mg of DPPH in 100 ml of Methanol. The 2 ml of the different concentrations of *C. odorata* extracts (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml) of water, ethanol, acetone and hexane were taken and 2 ml of the DPPH solution (0.135 mM) was added. The control sample was prepared by 2 ml of DPPH solution alone without sample mixed with 2 ml of methanol. Then all the test tubes were incubated in dark room for 30 min at room temperature. After the incubation period, the changes in the absorbance were measured at 517 nm in UV-Spectrophotometer. Methanol was used as blank to set zero in the UV-Spectrophotometer. The antioxidant activity was calculated in inhibition percentage by using following formula:

$$\text{Inhibition percentage} = \frac{[\text{Control O.D.} - \text{Sample O.D.}]}{\text{Control O.D.}} \times 100$$

ANTIBACTERIAL ACTIVITY:

The Disc Diffusion Method of Antimicrobial Susceptibility testing was used to evaluate the presence of antibacterial activities of the different solvent extracts of *C. odorata* assayed by the following method of Sasikumar *et al.* (2007). Two grams positive and two grams negative bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa*) were used for testing. The bacteria cultured overnight at 37°C in Nutrient broth. The sterile Muller Hinton Agar (Hi-media) plates were prepared. The 25 µg/ml 50 µg/ml and 100 µg/ml of the various solvent extracts were prepared by

using DMSO. Then 0.2 ml of the bacterial suspension was introduced into the sterile MHA plates and spreading the bacteria using L-Rod to get an even culture all over the plates. The 6 mm Discs was prepared from Whatman No.1 filter paper and autoclaved. A plate comprises of five discs, one is positive control, one is negative control and three different concentrations (25 µg/ml 50 µg/ml and 100 µg/ml) of the same plant extract. Where streptomycin is used as positive control and DMSO is used as negative control. The disc is placed with positive control, negative control and three different concentrations of the plant extracts of *C. odorata*, and then placed on the prepared Muller Hinton Agar plates. Then all the plates were kept for incubation at 37 °C for 12-18 h.

ANTI-HELMINTIC ACTIVITY:

The anti-helminthic properties of different solvent extracts of *C. odorata* were evaluated by following the method of Ghosh and Bhattacharya (2015). *Pheretima posthuma* (adult earthworm) which resemble both anatomically and physiologically to the human intestinal parasitic roundworms were used to evaluate the anti-helminthic activity. Each of the solvent extracts of *C. odorata* were dissolved to different concentrations (50 mg/ml and 100 mg/ml) with normal saline and evaluated for its efficacy against *P. posthuma*. One *P. posthuma* worm (3-5 cm length and 2-4 mm in width) were placed in each Petri dish containing 10 ml of each of the extracts, while Albendazole (50 mg/ml) and Normal saline served as positive and negative control, respectively. Each of the petri dishes with the earthworms were observed carefully and the time taken for paralysis (when no sort of movement observed even after vigorous shaking) and death (when dipped in water bath kept at 45 °C).

Results and Discussion

Fluorescence Analysis:

The ultra violet light produces fluorescence in several natural products, which do not noticeably fluoresce in daylight. If the substances themselves

are not fluorescent, they may often be rehabilitated into fluorescent derivatives or decomposition products by applying diverse reagents. Therefore, some crude drugs were often assessed qualitatively in this technique and it is an imperative parameter of pharmacological evaluation. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some show fluorescence in the visible range in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluations were done. The fluorescence analysis of leaf powder of *C. odorata* was carried out after treating with several solvents. Fluorescence efficiency using day light and UV light were carried out and represented with colour changes. Table 3 and Figures 2 and 3 show the variation in colour as observed also by Gupta *et al.* (2006), Ansari (2006) and Malathi *et al.* (2018).

Phytochemical Screening:

The phytochemical analysis of the various solvents extracts of *C. odorata* were tested for 13 phytochemicals and the results are shown in Table 4 and Figure 4. The procedure for the phytochemical analysis was carried out using standardized protocols (Harbone, 1973). The preliminary phytochemical screening of *C. odorata* leaves showed the presence of various secondary metabolites including alkaloids, tannins, flavonoids, saponins etc. (Table 4, Fig. 4) and this finding agrees with the report of Odutayo *et al.* (2017) and Fauzi *et al.* (2020) as they revealed the existence of saponins, terpenoids, flavonoids, tannins, and phenols in the leaf extracts of *C. odorata*. Phytochemical analysis of *C. odorata* leaves extracts revealed the presence of flavonoids and carbohydrate in all tested solvents. Tannins, saponins and glycosides are present in aqueous, ethanol and acetone extracts but absent in hexane extracts. The presence of tannins in our study

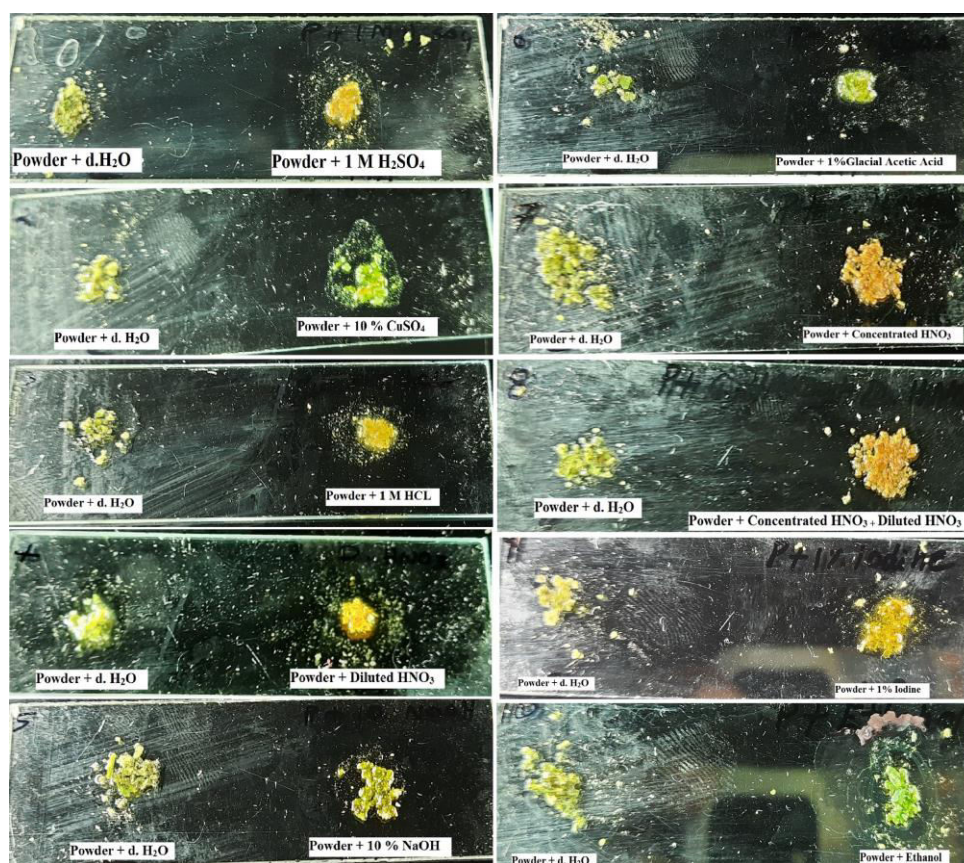
gives credence to the anti-bacterial effects of *C. odorata* on some known human pathogens such as *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* as reported by Mbajiuka *et al.* (2004) and Usunobun and Ewere (2016). The presence of saponins suggests that *C. odorata* may contain useful phytoconstituents that could reduce blood cholesterol level and cancer risk due to its foaming ability that produces soapy effect as reported by Okwu (2003) and Abubakar *et al.* (2020). The presence of alkaloids and flavonoids in *C. odorata* extracts is an indication of anti-inflammatory, antimalarial, and analgesic effects (Okwu and Josiah, 2006). It could be used to treat malaria, diabetes, cancer, cardiac dysfunction, stomach ache, relief pains, and gastro intestinal disorders. Flavonoids have been reported to have strong activity to change the body's reaction to allergies, virus and carcinogens, as they have anticancer activities, anti-allergic, and anti-inflammatory activities (Okwu, 2004). Oxalate was present in ethanol, acetone and hexane extract but not in aqueous extract. Coumarin was present in ethanol and acetone extracts and absent in aqueous and hexane. Phlobatanins and amino acids were present in aqueous extract alone and these compounds were absent in other tested solvents such as ethanol, acetone and hexane. Phenol molecule was only present in acetone extract and absent in all other tested solvents. Quinones were absent in all tested four solvents (Table 4, Fig. 4).

Antioxidant Activity:

The antioxidant activity of *C. odorata* extracts such as aqueous, ethanol, acetone and hexane were tested for antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method and it is compared with standard ascorbic acid (Fig. 5). Evidence collected in recent year suggested that the involvement of free radicals and other oxidants were the major cause of oxidative stress that lead to a variety of diseases and disorders. This led to an increasing interest in natural products having antioxidant properties. The use of DPPH scavenging assays in assessing the cell membrane integrity/cell membrane

Table 3: Fluorescence analysis of leaf powder of *C. odorata* in various reagents

Reagents	Colour under day light	Observation under UV
Powder + d. H ₂ O	Dark green	Dark green
Powder + 1 M H ₂ SO ₄	Dark green	Dark green
Powder + 10 % CuSO ₄	Yellowish Green	Violet
Powder + 1 M HCl	Fluorescent yellow	Violet
Powder + Diluted HNO ₃	Fluorescent yellow	Violet
Powder + 10 % NaOH	Dark Green	Pale Green
Powder + 1% Glacial Acetic Acid	Green	Pale Green
Powder + Concentrated HNO ₃	Brownish yellow	Pale Brown
Powder + Conc. HNO ₃ + Dil.HNO ₃	Brownish yellow	Pale Brown
Powder + 1% Iodine	Fluorescent yellow	Pale Green
Powder + Ethanol	Dark Green	Green

Fig. 2: Fluorescence analysis of leaf powder of *C. odorata* under day light.

stabilizing capacities of plant constituents has given explanations as to the possible ways by which phytomedicine could help to reduce diseases caused by infections, inflammation and oxygen radical generation affecting the cell membrane (Shahidi and Wanasundara, 1992).

Plants have been considered as rich sources of antioxidants. In our study the antioxidant activity of *C. odorata* solvents extracts were high in the ethanol followed by aqueous, acetone and hexane and the O.D. value of the tested samples were used to calculate the Percentage of Inhibition and



Fig. 3: Fluorescence analysis of leaf powder of *C. odorata* under UV light.

Table 4: The phytochemical analysis various organic extract of *C. odorata*

S. No.	Phytochemical Tests		Aqueous	Ethanol	Acetone	Hexane
1.	Alkaloids	Wagner's test	+	-	-	+
2.	Amino Acid	Xanthoprotein test	+	-	-	-
3.	Carbohydrate	Benedicts test.	+	+	+	+
4.	Phenol	FeCl ₃ test.	-	-	+	-
5.	Flavanoids	Alkaline reagent test	+	+	+	+
6.	Tannins	FeCl ₃ test.	+	+	+	-
7.	Saponin	Foam test.	+	+	+	-
8.	Terpenoids	Salkowski's test.	+	-	-	+
9.	Phlobatanins	1% HCl test	+	-	-	-
10.	Quinones	Hydrochloric Acid test	-	-	-	-
11.	Coumarin	Sodium hydroxide test	-	+	+	-
12.	Oxalate	Glacial acetic acid test	-	+	+	+
13.	Glycoside	Killarkillani's test	+	+	+	-



Fig.4: The phytochemical analysis various organic extract of *C. odorata*.

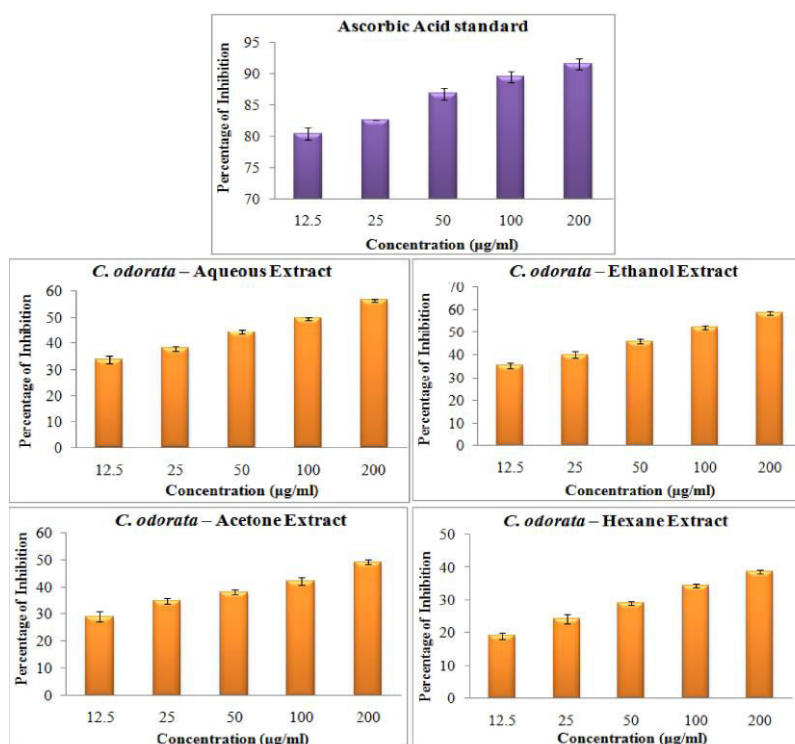


Fig. 5: DPPH radical scavenging activity of *C. odorata* leaves extracts.

Table 5: Antibacterial activity of *C. odorata* against various bacterial pathogens

Bacteria	Solvents	Zone of Inhibition in mm				
		Negative Control (DMSO)	Positive Control (10 µg)	25 µg	50 µg	100 µg
<i>B. subtilis</i>	Aqueous	-	15	5	7	9
	Ethanol	-	14	5	10	12
	Acetone	-	13	6	8	10
	Hexane	-	13	0	0	0
<i>S. aureus</i>	Aqueous	-	6	0	0	0
	Ethanol	-	7	0	6	7
	Acetone	-	7	0	5	5
	Hexane	-	7	0	0	4
<i>E. coli</i>	Aqueous	-	6	0	0	0
	Ethanol	-	6	0	0	5
	Acetone	-	5	0	0	5
	Hexane	-	6	0	4	6
<i>P. aeruginosa</i>	Aqueous	-	6	0	0	0
	Ethanol	-	5	4	4	5
	Acetone	-	8	4	4	7
	Hexane	-	7	7	7	7

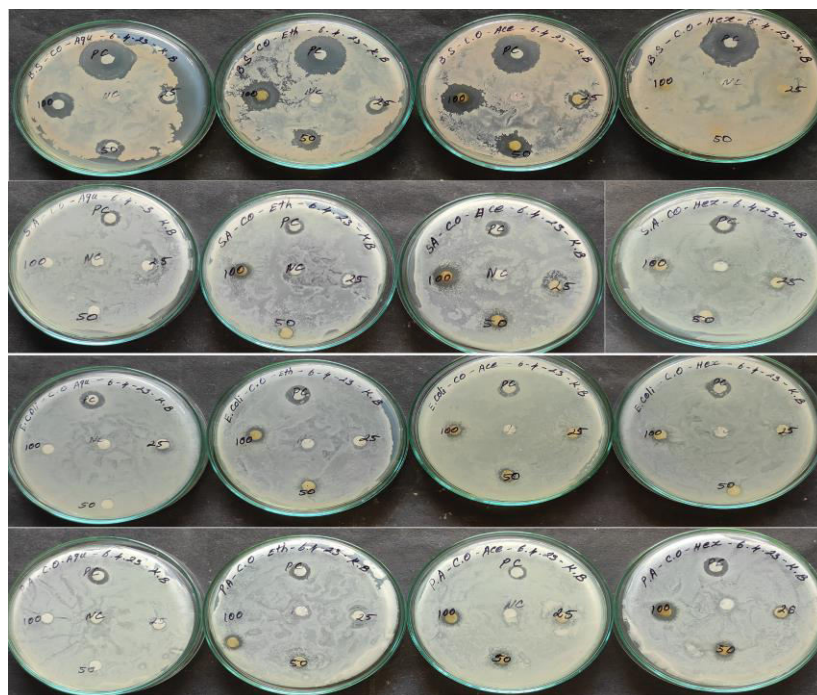
Fig. 6: Antibacterial activity of *C. odorata* against various bacterial pathogens.

Table 6: Anti-helminthic activity of leaf extracts of *C. odorata*

Group	Treatment	Concentration used (mg/ml)	Time taken for paralysis (min.)	Time taken for death (min.)
1	NC - Normal Saline	-	-	-
2	PC - Albendazole	50	8.00 ± 0.58	13.33 ± 0.33
2	PC - Albendazole	100	5.33 ± 0.33	8.33 ± 0.33
3	Aqueous	50	542.33 ± 12.20	594.33 ± 12.13
4		100	498.33 ± 10.14	531.67 ± 5.49
5	Ethanol	50	59.00 ± 2.08	92.33 ± 3.71
6		100	10.00 ± 0.58	13.00 ± 1.15
7	Acetone	50	668.33 ± 4.41	679.00 ± 2.65
8		100	493.33 ± 8.82	509.00 ± 2.08
9	Hexane	50	722.67 ± 11.85	657.00 ± 4.04
10		100	537.00 ± 2.52	565.33 ± 3.38

Fig. 7: Anti-helminthic activity of leaf extracts of *C. odorata*.

Standard Error. Percentage of Inhibition and Standard Error are expressed in Figure 5. The result of reducing power ability of *C. odorata* as shown in Figure 5 exhibit that leaves has a dose-dependent effect as the reducing power ability of the plant increased with increasing concentrations from 12.5 µg/ml to 200 µg/ml as reported by Usunobun and Ewere (2016).

Antibacterial Activity:

The Disc Diffusion Method (Mostafa *et al.*, 2018) for Antimicrobial Susceptibility testing was used to evaluate the presence of Antibacterial Activities of the different solvent extracts of *C. odorata*. *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* were used for the testing. The bacteria were cultured overnight at 37°C in Nutrient broth.

The sterile Muller Hinton Agar (Hi-media) plates were prepared. The 25 µg/ml, 50 µg/ml and 100 µg/ml of the aqueous, ethanol, acetone and hexane solvent extracts were prepared by using the DMSO. Then 100 µl of the bacterial suspension was introduced into the sterile plates and spreading the bacteria using L-Rod to get an even culture all over the plates. The 6mm Discs was prepared from Whatman No.1 filter paper and autoclaved. A plate comprises of five discs, one is positive control, one is negative control and three for the three different concentrations (25 µg/ml, 50 µg/ml and 100 µg/ml) of the same plant extract. The sterile discs are placed above the media and the samples are loaded gently by the following quantity given below. Where 10 µl of Streptomycin (10 µg/ml) was loaded as positive control in the disc and 10 µl of DMSO alone used as negative control in the disc. Then 10 µl of the 25 µg/ml, 50 µg/ml and 100 µg/ml prepared concentration for the plant extracts were loaded gently in the respective discs. Then all the plates are kept for incubation at 37°C for 24 h. The antibacterial activity of *C. odorata* extracts correlated with the amount of both total phenol and flavonoid compounds. The results obtained suggests that the ethanolic and acetone leaf extracts are potential natural source for drug development to treat bacterial infections which is in agreement with findings of Vijayaraghavan *et al.* (2018). Different levels of inhibitions against test organisms at concentration of 25, 50 and 100 µg/ml of all the solvent extracts were observed and depicted in Table 5 and Figure 6. The results indicated the activity of extracts against all the tested clinical bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. These findings agree with the report of Abubakar *et al.* (2020).

Anti-Helminthic Activity:

The present study revealed that the *C. odorata* ethanol extract was endowed with potent anti-helminthic property as compared to other extracts such as aqueous, acetone and hexane, but these extracts also possess significant activity as reported by Debashisha *et al.* (2010) (Table 6, Fig.

7). The extracts were also found to be lethal to the worms up to the concentration of 100 mg/ml of ethanol extract. This anti-helminthic activity was comparable with the reference drugs, Albendazole. The above findings justified the anti-helminthic properties of the plant as suggested by the folklore practice.

Conclusion

Plants are the basic and traditional source of knowledge for modern life sciences especially in medicine. The relatively lower incidence of adverse reactions to plant preparations, compared to modern conventional pharmaceuticals, coupled with their reduced cost is encouraging for both the consuming by public and national health care institutions to consider plant medicines as alternative to synthetic drugs. With this preliminary research studies, we conclude that the plant species *C. odorata* found to be a good source of various bioactive compounds and it has good phytochemical and pharmacological activity. With these studies we can further isolate the active compounds present in it with various parameters like FTIR, HPLC, GC-MS etc., and further can analyze the isolated compounds for *in vitro* assays to study the various activities by using various cell lines studies which can leads for the research and development of various nutritional and medicinal value-added products from *C. odorata* in near future.

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References

- Abubakar MA, Etonihu AC, Kigbu PE, Owuna JE and Audu SI. (2020) Phytochemical and antimicrobial analyses of leaf extracts of *Cerathoteca sesamoides* and *Chromolaena odorata*. Int J Res – GRANTHAALAYAH, 8(08): 65 -74.
- Akinmoladun AC, Ibukun EC and Dan Ologe IA. (2007) Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. Sci Res Essays 2: 191-194.

- Ansari SH. (2006) Essentials of Pharmacognosy. 1st edn., Birla Publications Pvt. Ltd, New Delhi.
- Chang CC, Yang MH, Wen HM and Chern JC. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10(3): 178-182.
- Debashisha P, Dash SK and Dash GK. (2010) Qualitative Phytochemical analysis and investigation of anthelmintic and wound healing potentials of various extracts of *Chromolaena odorata* Linn. collected from the locality of Mohuda Village, Berhampur (South Orissa). *Int J Pharmaceut Sci Rev Res* 1(2): 122-126.
- Fauzi NAM, Tan NFMS, Razak AHA and Mansur SA. (2020) Qualitative phytochemical analysis and antibacterial potential of *Chromolaena odorata* leaves as affected by Soxhlet and maceration extraction. *J Adv Indust Technol Application* 1(2): 38-48.
- Ghosh K and Bhattacharya TK. (2015) Chemical constituents of *Piper betle* Linn. (Piperaceae) roots. *Molecules* 10: 798-802.
- Gupta MK, Sharma PK, Ansari SH and Lagarkha R. (2006) Pharmacognostical evaluation of *Grewia asiatica* fruits. *Int J Plant Sci* 1(2): 249-261.
- Harborn JB. (1973) Phytochemical methods: A guide to modern techniques of plants analysis. Chapman and Hall London, Ltd.
- Heywood VH, Harborne JB and Turner BL. (1977) Biology and chemistry of the Compositae. 1. Academic Press, London.
- Kouamé PB, Jacques C, Bedi G, Silvestre V, Loquet D, Barillé-Nion S, Robins RJ and Tea I. (2013) Phytochemicals isolated from leaves of *Chromolaena odorata*: Impact on viability and clonogenicity of cancer cell lines. *Phytother Res* 27: 835-840.
- Ling SK, Pizar MM and Man S. (2007) Platelet-activating factor (PAF) receptor binding antagonist activity of the methanol extracts and isolated flavonoids from *Chromolaena odorata* (L.) King and Robinson. *Biol Pharm Bull* 30: 1150-1152.
- Malathi R, Kaviyaranan D and Chandrasekar S. (2018) Preliminary phytochemical analysis of *Justicia adhatoda* leaves extract using different solvents. *Int J Pharmaceut Drug Anal* 6(2): 186-190.
- Mbajiuka CS, Obeagu EI, Chude CN and Ihezue OE. (2004) Antimicrobial effects of *Chromolaena odorata* on some human pathogens. *Int J Curr Microbiol Appl Sci* 3(3): 1006-1012.
- Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN and Bakri MM. (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J Biol Sci* 25: 361-366.
- Mukherjee KP. (2002) Quality control of herbal drugs – An approach to evaluation of botanicals. Business Horizons, pp. 816.
- Odutayo F, Ezeamagu C, Kabiawu T, Aina D and Mensah-Agyei G. (2017) Phytochemical screening and antimicrobial activity of *Chromolaena odorata* leaf extract against selected microorganisms. *J Adv Med Pharmaceut Sci* 13(4): 1-9.
- Okwu DE. (2003) The potentials of *Ocimum gratissimum*, *Penrularia extensa* and *Tetrapleura tetraptera* as spice and flavouring agents. *Nigerian Agricult J* 34: 143-148.
- Okwu DE. (2004) Phytochemicals and vitamin content of indigenous spices of southeastern Nigeria. *Nigerian J Sustainable Agricult Environ* 6(1): 30-37.
- Okwu DE and Josiah C. (2006) Evaluation of the chemical composition of two Nigerian medicinal plants. *African J Biotechnol* 5(4): 357-361.
- Olawale F, Olofinisan K and Iwaloye O. (2022) Biological activities of *Chromolaena odorata*: A mechanistic review. *S Afr J Bot* 144: 44-57.
- Phan TT, Wang L, See P, Grayer RJ, Chan SY and Lee ST. (2001) Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biol Pharm Bull* 24: 1373-1379.
- Sasikumar JM, Thayumanavan T, Subashkumar R, Janardhanan K and Lakshmanaperumalsamy P. (2007) Antibacterial activity of some ethnomedicinal plants from the Nilgiris, Tamil Nadu, India. *Natural Product Radiance* 6: 34-39.
- Shahidi F and Wanasundara PK. (1992) Phenolic antioxidants. *Crit Rev Food Sci Nutr* 32(1): 67-103.
- Taleb-Contini SH, Kanashiro A, Kabeya LM, Polizello AC, Lucisano-Valim YM and Oliveira DC. (2006) Immunomodulatory effects of methoxylated flavonoids from two *Chromolaena* species: Structure-activity relationships. *Phytother Res* 20: 573-575.
- Touré D, Kouamé B, Bedi G, Joseph A, Guessennd N, Oussou R, Chalchat JC, Dosso M and Tonzibo F. (2014) Effect of geographical location and antibacterial activities of essential oils from Ivoirian *Chromolaena odorata* (L.) RM King & Robinson (Asteraceae). *J Pharmacogn Phytother* 6: 70-78.
- Usunobun U and Ewere GE. (2016) Phytochemical analysis, mineral composition and in vitro antioxidant activities of *Chromolaena odorata* leaves. *ARC J Pharmaceut Sci* 2(2): 16-20.
- Vijayaraghavan K, Rajkumar J and Mohamed Ali S. (2018) Phytochemical screening, free radical

- scavenging and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic bacterium in wound infections– a multispectrum perspective. *Biocatalysis Agricult Biotechnol.* 15: 103-112.
- Vijayaraghavan K, Rajkumar J and Seyed MA. (2017) Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. *Vet Med.* 62: 565-578.
- Vijayaraghavan K, Rajkumar J, Bukhari SN, Al-Sayed B and Seyed MA. (2017) *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities. *Molec Med Rep.* 15(3): 1007-1016.
- Vital PG and Windell LR. (2009) Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts. *J Med Plants Res.* 3: 511-518.
- Wang L, Waltenberger B, Pferschy-Wenzig EM, Blunder M, Liu X, Malainer C, Blazevic T, Schwaiger S, Rollinger JM and Heiss EH. (2014) Natural product agonists of peroxisome proliferator-activated receptor gamma (PPAR γ): A review. *Biochem Pharmacol.* 92: 73-89.
- Warea O. (2004) *Chromolaena* (Siam) weed. Pest Advisory Leaflet no. 43. Secretariat of the Pacific Community.