EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 10
18-23
Year: 2019

PHYTOCHEMICAL AND ANTIFUNGAL PROPERTIES OF CHROMOLAENA ODORATA (SIAM WEED) LEAVES

[*1]Anyadoh-Nwadike Sylvia O., [1]Ahumibe N. C., [1]Manunyewor Donatus J. and [2]Ilo Cajetan Elochukwu

[1]Department of Biotechnology, Federal University of Technology, Owerri, Imo State, Nigeria.
[2]Department of Pharmacology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

***Corresponding Author: Anyadoh-Nwadike Sylvia O.**
Department of Biotechnology, Federal University of Technology, Owerri, Imo State, Nigeria.

Article Received on 27/06/2019 **Article Revised on 16/07/2019** **Article Accepted on 06/08/2019**

ABSTRACT
Antifungal and phytochemical properties of hot water and ethanolic extracts of *Chromolaena odorata* were assayed using standard techniques. This was done to ascertain the susceptibilities of the challenge fungi to crude *C. odorata* extracts using two extraction methods and assess the active phytochemical constituents of the plant. Plant materials (leaves) were collected from Federal University of Technology, Owerri, Nigeria compound. The leaves were appropriately prepared and extraction of crude ingredients done using ethanol and hot water respectively under standard laboratory conditions. Nystatin (10 mg/dl) was also used for comparative purposes. *Candida albicans*, *Penicillium notatum*, *Geotrichum* sp., *Aspergillus niger* and *Aspergillus oryzae* were used as the challenge test organisms. Phytochemical analysis revealed the presence of bioactive flavonoids, polyphenols, saponins and tanins. Results revealed that all the test fungi were susceptible to both the hot water and ethanolic extracts observed as inhibition of growth at 100 mg/ml and 50 mg/ml respectively. *Aspergilus niger*, *Candida albicans* and *Aspergilus oryzae* showed the highest susceptibility to both the aqueous and ethanol extracts whereas *Geotrichum* sp and *Penicillium notatum* showed some level of resistance to bioactivity of both the aqueous and ethanol extracts. However, they were all susceptible to Nystatin (10 mg/dl). The presence of bioactive substances may be responsible for the antifungal activities of the extract. This study therefore underscores the fact that *C. odorata* can be a viable and cheap source for antifungal formulations though the efficacious dosage may be higher than that of Nystatin.

KEYWORDS: phytochemical, antifungal, *Chromolaena odorata*, Siam weed, ethanolic extracts, bioactive agents.

INTRODUCTION
Background: *Chromolaena odorata* (Siam weed) of the family *asteraceae* (compositae). It is a native of central and South America but has spread throughout the tropical and subtropical areas of the world (Vital and Windell 2009). It is a perennial, diffuse and scrambling shrub which grows to 3 to 7 m in height when growing in the open. It is now a major weed that is widespread in central and western Africa, tropical America, west India and south East Asia and western part in Nigeria (Koirala *et al*. 2005). It thrives in most soils and is a prolific weed found in abundance on open waste land and along road sides (Omokhua *et al.* 2015; Naidoo *et al.* 2001; Grierson and Afolayan 1999). It is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antimicrobial, antihypertensive, anti-inflammatory, astringent and hepatotropic agent (Phan *et al.* 2001; Akinmoladun *et al.* 2007; Naidoo *et al.* 2011; Omokhua *et al.* 2015).

The plant has also been claimed to have wound healing and diuretic activities (Sirinthipaporn 2017; Thang *et al*. 2001). It is also claimed to be applied tropically as an antidote against the sting from the spine of the common sea catfish (Chakraborty *et al*. 2011). An aqueous decoction of the roots is used as an antipyretic and analgesic remedy and a leaf extract with salt is used as a gargle for sore throats and colds. Fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Thang *et al*. 2001). A poultice of leaves is traditionally applied onto cuts or wounds to stop bleeding and promote healing (Vaisakh and Anima Pandey 2012). An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds (Bassey *et al.*, 2013). The aqueous and decoction of the leaves is used for the treatment of the soft wounds, burn wounds and

skin infections (Vaisakh and Anima Pandey 2012; Sirinthipaporn 2017).

Fungi are diverse group of microorganisms that have adapted themselves to live in a variety of environments and are quite distinct from bacteria in size, cellular structure and chemical composition however they considered 'higher beings' compared to bacteria (Stevens *et al*. 2000). Yeast-like fungi typically round or oval, generally form smooth flat colonies when growing in the laboratory, and reproduce by buds projecting from the mother cell. Molds are composed of tubular structures called hyphae and grow by branching thus producing extensions from the mother cell (Pappas *et al*. 2004). Fungi produce diverse human infections ranging from superficial skin infections to internal organ invasion in the body (e.g Candida infection of the mouth, vagina, throat and oesophagus) (Pappas *et al*. 2004). Common pathogenic fungi such as *Geotrichum, Penicillium, Candida* and *Aspergillus* species are among the major infectious agents in human. *C. albicans* is a commensal pathogen as it is a member of the gastrointestinal, oropharyngeal and female genital flora (Liu, 2004). It is also regarded as an opportunistic pathogen in humans as it can cause disease in immunodeficient and immunocompromised individuals that can be life threatening (Ruhnke 2002; Mayer *et al.* 2013). These infections usually occur as a result of a decrease in natural human defenses or opportunistic heavy exposure to the fungus. Therapies for these infections vary according to the type of infection. Superficial infections are often treated with topical agents whereas organ involvement must be treated with systemic therapy (Stevens *et al*. 2000).

Fungi are ubiquitous in the environment and have over the years, fungal infections have continued to scourge man. Infections due to fungal pathogens have continued to emerge and reemerge irrespective of many treatment regimens that have been used to curb the fungal infection. Many fungi have become resistant to known antifungal chemotherapy even from broad spectrum antifungal agents as reported by Dennis *et al.* (1996) underscoring the need for novel antifungal agents (Obi *et al*. 2011). Plants come in handy as easy sources of active antimicrobial agents (Okwu, 2004). However, according to Vital and Windell (2009), plants must be assessed from a scientific view point in order for them to be understood and reach their rightful role in contributing to affordable health care. Successive solvent extraction, chromatography separation and spectroscopy method are used to determine the chemical constituents of plant and to know the bioactivities in traditional medicine (Vital and Windell 2009). A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. Driven by the above information and the need for further exploitation of wild plant for possible novel antimicrobial substances, this study was therefore aimed at screening for the active principles or ingredients as well as possible antifungal properties of *Chromolaena odorata* leaves collected from Federal University of Technology Owerri, Imo State premises for possible isolation / identification of novel antimicrobial substances.

METHODOLOGY

STUDY AREA

The study was conducted at the department of Biotechnology, Federal University of Technology, Owerri, Imo state in the South-Eastern part of Nigeria. It is located within the tropical Rain forest belt of Nigeria. This tropical rainforest boasts of lush vegetation of which *C. odorata* is among the prominent weeds.

COLLECTION AND PREPARATION OF PLANT MATERIAL

Fresh leaves of *C. odorata* were collected from the wild around School of Biological Science, Federal University of Technology Owerri premises. The plant was identified as *Chromolaena odorata* by a botanist in the university. The plant leaves were cleaned and air dried at room temperature till crispy, for fourteen days. The dried leaves were then pulverized with an electric blender, and the powder transferred into a sterile glass container for future use.

COLLECTION AND PREPARATION OF TEST FUNGI

Stock cultures of *Geotrichum sp., Aspergillus niger, Aspergillus oryzae* and *Penicillium notatum* were obtained from the Department of Biotechnology laboratory, Federal University of Technology Owerri, Imo state, Nigeria while *Candida albicans* was sourced from the laboratory unit of a hospital. The fungi were respectively subcultured on sterile solid Sabourand Dextrose Agar (SDA) medium using the methods described by Patil and Shettigar, (2010). Pure cultures were obtained after 48 to 72 hrs and stored at 4^{o}C for further use. The pure cultures were further confirmed using the methods described by Cheesbrough (2004) and Tsuneo (2010).

EXTRACTION OF BIOACTIVE SUBSTANCES

The extraction of active ingredients from *C. odorata* was carried out as described by AOAC, (2002) using ethanol and hot water respectively.

Ethanol extraction was done using Soxhlet extractor. Fifty grams (50 g) of the blended leaves were weighed out and transferred into the thimble. The thimble was then inserted into the soxhlet extraction chamber. The extraction was done for 24 hrs with 200 ml of absolute ethanol. The extracts obtained were concentrated, allowed to cool, poured into appropriately labeled sterile bottle, and stored for future use.

For hot water extraction (aqueous), Twenty grams (20 g) of the blended leaves were weighed out using a weighing balance. The weighed plant material was boiled in 100

ml of distilled water for 1 hr. The solution was allowed to cool and filtered into a sterile bottle, labeled and stored appropriately for further use.

PHYTOCHEMICAL SCREENING

The phytochemical screening followed the protocols as described by Harbone (1973) and Duru *et al*. (2012). The following bioactive substances were screened for; Alkaloids, saponins, tannins, cardiac glycosides, polyphenols, flavonoids and terpenoids.

DETERMINATION OF ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *C. odorata*

The minimum inhibitory concentration **(**MIC) and the minimum fungicidal concentration (MFC) were both determined. These were done using standard microbiological procedure as described by Cheesbrough (2004).

The MIC was determined as the lowest concentration at which no growth occurred in malt extract medium i.e. the lowest concentration of crude plant extract in the broth medium that inhibited visible growth of the test fungal strains. Different concentrations of the crude extract were achieved with the broth medium as follows: 25 mg/dl, 50 mg/ml, 100 mg/ml and 200 mg/ml. Commercially available 10 mg/ml of nystatin was also added to a separate malt extract broth medium and used for comparative purposes. Each assay was carried out in triplicates respectively. The MIC test was used to quantify the antifungal activity of the plant extract.

Minimum fungicidal Concentration (MFC) of the crude extracts was also respectively determined using the methods of Baron and Finegold (1990).

This was done by first selecting tubes that showed no growth during MIC determination. About 20 µl from each of the selected tubes was sub-cultured onto drug/extract free malt extract agar plates and incubated for further 3 - 7 days at 27°C. The least concentration, that showed no growth in the subculture was observed and noted as the minimum fungicidal concentration (MFC).

The MFC was regarded as the lowest extract concentration that did not yield any fungal growth on the solid medium used.

RESULTS

Table 1: Result of the Phytochemical Screening

TEST	RESULT
Alkaloids	+ve
Tannins	+ve
Saponins	+ve
Flavonoids	+ve
Terpenoids	-ve
Cardiac glycosides	-ve
Polyphenols	+ve

Legend: +ve = Present; -ve = Absent

Table 2: Minimum Inhibitory Concentration (MIC) Test Result (mg/ml).

Organisms Extract	*Penicillium notatum*	*Geotrichum sp.*	*Aspergillus oryzae*	*Candida albicans*	*Aspergillus niger*
Ethanolic	100	100	50	50	50
Aqueous	_	_	100	50	50
Nystatin (10mg/ml)	-ve	-ve	-ve	-ve	-ve

Legend: +ve= Visible growth; -ve= No growth ; _ = Growth in all concentrations

Table 3: Minimum Fungicidal Concentration (MFC) Test Result (mg/ml)

Organisms Extract	*Penicillium notatum*	*Geotrichum sp.*	*Aspergillus oryzae*	*Candida albicans*	*Aspergillus niger*
Ethanolic	100	100	50	50	50
Aqueous	_	_	100	100	100
Nystatin (10mg/ml)	-ve	-ve	-ve	-ve	-ve

Legend: +ve = Visible growth; -ve = No growth; _ = Growth in all concentrations

DISCUSSION

The phytochemical screening results showed that the plant material (*C. odorata*) possess the following active ingredients, flavonoids, polyphenols, saponins and tannins as shown in Table 1. Nihu, (2010), had earlier reported the presence of flavonoids, monoterpenes, steroids, phenolic compounds, etc. King *et al.* (2017) and Anyasor *et al.* (2011), also reported the presence of alkaloids, saponins, cardiac glycosides, flavonoids, tannins, phlobatannins, steroids and terpenoids in *C. odorata* parts However, the present study showed absence of Terpenoids and cardiac glycosides. Igbo *et al*. (2009) had also reported presence of the bioactive substances in *C. odorata* obtained from Port Harcourt, Rivers State. However, they also found cardiac glycosides (though in low percentage; 0.05% ww) which were absent in the current study. This study generally aligned with the fact that *C. odorata* may habour potential bioactive substances of clinical importance. The phenolic compounds and derivatives present have been

reported as major and powerful antioxidants to protect cultured skin cells against oxidative damage (Thang *et al.* 2001) and other clinical importance (Hassing *et al.* 1999). The crude ethanol extract of the plant has been demonstrated to be a powerful antioxidant to protect fibroblasts and keratinocytes *in vitro* (Mahmoud *et al.* 2011). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins (Anyasor *et al.* 2011) as corroborated by the present study. In recent past, attention has been directed to medicinal research to substantiate the claims of cure made by the traditional healers and thus provide a scientific basis for their efficiency (Olukoya *et al.* 2003).

The antifungal activity results showed that the inhibitory effects were dependent on the concentration, solvent used for the extraction of plant material alongside the phytochemical properties of the extract (active ingredients) as shown in Table 2. The result of the antimicrobial activities of the extracts showed the test fungi; *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium notatum*, *Candida albicans* and *Geotrichum* species were generally susceptible to the test plant extract especially the ethanolic extracts. This implies that the ethanolic extracts of *C. odorata* are potential agents to be used against the test organisms. Formulations from it will be efficacious against the fungi at the in-use concentrations.

The MIC value was in the range of 50 mg/ml and 100 mg/ml with the lowest (50 mg/ml) for all the ethanolic extract. The ethanolic extract showed higher antifungal effect on the test fungi than the aqueous extract (Table 2). This could be due to the fact that the active ingredients dissolved more readily in ethanol possibly due to its volatility as reported by Adedeji *et al*., (1991). The MIC and MFC of the ethanolic extracts seem to be same for the respective fungus (Tables 2 and 3). This was different for the aqueous extracts where the MFC was higher than the MIC for three of the test fungi (Tables 2 and 3). It is worthy to note that at concentrations as high as 200 mg/dl, the aqueous extracts showed no sign of inhibition of both *P. notatum* and *Geotrichum* sp. The aqueous extracts of *C. odorata* will therefore not be potential clinical agents against these two fungal species.

Both the ethanolic and aqueous extracts showed the highest inhibitory activity at 50 mg/ml on *Candida albicans* and *Aspergillus niger*. Iwu (2003) had earlier shown that crude extract of *C. odorata* has antifungal effect on *Aspergillus niger* corroborating the result of this study. The MIC and MFC of the ethanolic and aqueous extract were similar for the moulds and yeast used in this study. This shows that the crude extract has a broad spectrum of activity corroborating the report of Kar, (2009).

All fungal isolates were susceptible to Nystatin thereby validating it as a broad spectrum antifungal agent and as a valid positive control. Comparative activity was recorded for the crude extract with common conventional fungicide Nystatin as the cidal action was similar to the MFC of the crude extract used. Though the crude extracts showed activity at a higher concentration (50 mg/dl and 100 mg/dl respectively) than the common conventional fungicide Nystatin (10 mg/dl), both still showed similar fungicidal activity especially the ethanolic extracts (Tables 2 and 3). This shows that the plant extract could be standardized and used as a possible source of antimicrobial formulation. Anyasor *et al.*, (2011) have earlier noted that toxicity of *Chromolaena odorata* to microorganism is probably due to the relative presence of the different toxic phytochemicals. This has been corroborated by this study as the antifungal activities may be as a result of the bioactive substances/ phytochemicals present in the test plant (Table 1).

CONCLUSION

This study has revealed that ethanolic and aqueous extract of *Chromolaena odorata* (Siam weed) contains active phytochemicals such as flavonoids, polyphenols, saponins and tannins. These active ingredients may be responsible for the activity of the crude extract on the fungi. This was especially shown by the ethanolic extract which showed both -static and -cidal actions against all the test fungi at varying concentrations.

From the foregoing, it is evident that the *C. odorata* extracts can be formulated for use against diseases caused by any of the test fungi. This goes further to corroborate the opinion of Okwori *et al*., (2007), that Herbal medicine is readily available in our diverse vegetation, cheap, and carries the potential of introducing new templates into modern medicine. The degree of efficacy may however be below the standard antibiotic used in this study as positive controls and thus requires further investigation and standardization processes.

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