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PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSES OF LEAF EXTRACTS OF CERATHOTECA SESAMOIDES AND CHROMOLAENA ODORATA

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ABSTRACT

It was reported in 2005 during WHO survey that about 70-80% of the world population use medicinal plants either in their crude unmodified form or partially in their modified semi-synthetic form of plant sources in their primary healthcare. The present study investigated the phytochemicals and antimicrobial activities of the leaf extracts of Cerathoteca sesamoides and Chromolaena odorata to ascertain their potentials in herbal medicine. Fresh leaf of the plants obtained from Lafia in Nasarawa State, Nigeria were dried, powdered, and subjected to methanolic extraction, partition, phytochemical, and antimicrobial analyses using standard methods. Partitions from n-hexane, methanol, ethyl acetate, chloroform, and residue extracts were tested against clinical bacteria Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus, and fungus Candida albicans. Among the four different solvents used in partitioning methanolic and ethyl acetate extracts of both plants contain flavonoids, tannins, alkaloids, terpenoids, saponins, steroids, and cardiac glycosides. Saponins were absent in the n-hexane and chloroform extracts of C. odorata and the ethyl acetate extract of C. sesamoides. While flavonoids were present in the n-hexane extracts of C. odorata, they were absent in C. sesamoides. Anthraquinone and reducing sugar were absent in all the solvent extracts of both medicinal plants. The antimicrobial susceptibility tests showed that n-hexane and residue extracts of both plants had no activity against the tested microorganisms. The chloroform and ethyl acetate extracts of C. sesamoides and C. odorata (at 12.5 mg/ml) were active against all the tested clinical bacteria K. spp., E. coli, P. aeruginosa, S. aureus, and C. albicans. The methanolic extracts of both plants were active against the bacterial isolates but inactive against C. albicans. The minimum bactericidal concentration of these plant extracts was ≥50 mg, while the minimum inhibition concentrations ranged between 12.5 mg and ≥50 mg. The findings showed that the chloroform or ethyl acetate extracts of the leaves of these plant drugs could be used to treat urinary tract infections.

1. INTRODUCTION

In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from difference sources (Mukeshwar *et al.,* 2011). Today, it has been developed as a separate industry as many people prefer herbal medicines to synthetic ones. Over 80,000 species of different plants are in use throughout the world, but more than 500 traditional communities use about 800 species of plants for curing different diseases (Kamboj, 2000). WHO survey in 2005 also reported that about 70-80% of the world population, particularly in the developing countries, depend on non-conventional medicines mainly of plant sources in their primary healthcare. Therefore, WHO has described traditional medicine as one of the surest means to achieve total healthcare coverage of the world's population. In Nigeria, variations can be observed in ethnic names and use of local biodiversity indicating the intimate and independent usages of local resources (Stella *et al*., 2000). These local resources include the use of plants for treatment of diseases, livestock fodder, human food and nutrition, construction and firewood. Plants used in the treatment of diseases contain active compounds which are phytochemicals with biological activities. Some of which are responsible for the characteristic odours, pungencies and colours of plants while others give a particular plant its culinary, medicinal or poisonous virtues (Evans and Trease, 2002).

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in Nigeria contain at least one plant-derived ingredient. Various traditional medicine practices have been developed in different cultures, different regions, but without a parallel development of international standards and appropriate ways for evaluating them (WHO, 2005). WHO has encouraged the rational use of plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicines (WHO, 2000).

Ceratotheca sesamoides, a member of the *Pedaliaceae* family, is a flowering plant of the genus *Ceratotheca*. It is indigenous to Africa and grows both as a wild weed and locally cultivated species, and is colloquially referred to as false sesame owing to its marked similarities with common sesame (*Sesamum indicum*). *Ceratotheca sesamoides* is a plant with many practical uses and applications in food and medicine (Toyin *et al.,* 2012; Adesiyun and Uddin, 2011; Bedigian and Adetula, 2004; Fasakin, 2004). The leaves and flowers are often consumed as vegetables or used in sauces. The leaves also have medicinal benefits while the seeds can produce cooking oil. Aqueous leaf extracts are used in the treatment of diarrhea, dysentery, and measles. The leaf may be an effective anti-oxidant, anti-inflammatory and anti-hypertensive agent, while the mucilage can be used as an emollient and lubricant. The slimy liquid produced by soaking the leaves in water can be used to treat conjunctivitis (Adesiyun and Uddin, 2011). Warm leaves can be ground and mixed with ash then applied to inflamed cervical lymph nodes to help expedite delivery in both humans and animals. If the leaves are ground with the rhizome of *Anchomanes difformis,* the ensuing mixture has been used to treat cases of leprosy. False sesame has been claimed to possess some anti-viral properties and has been employed as an aphrodisiac against jaundice, snakebites and skin diseases (Bedigian and Adetula, 2004). The seed oil is similar in composition to sesame oil and contains sesamin, a phenyl propanoid lignin. This compound showed anti-oxidant, anti-inflammatory, anti-hypertensive, cytotoxic (including anti-tumour) and insecticidal activities (Phan *et al*., 2001).

Chromolaena odorata, a known toxic weed from the family of *Asteraceae,* is widespread over many parts of the world, including Nigeria. The weed is known with many common names including "Awolowo", in Igbo language, "Obirato" in Bumaji-Boki, siam weed, bitter bush or jack in the bush (Okon and Amalu, 2003). *Chromolaena odorata* is also called Independence leaf, as its popularity coincided with Nigeria's Independence in 1960. In traditional medicine, it is used as an anti-spasmodic, anti-protozoal, anti-trypanosomal, anti-bacterial, anti-fungal, anti-hypertensive, anti- inflammatory, astringent, diuretic and hepatotropic agent (Phan *et al.*, 2001). In Ivory Coast, the plant is exploited for its anti-inflammatory properties, which may be due to the terpenes present, which show excellent inhibition of soya lipoxygenase L-1 (Owoyele *et al.,* 2005; Bedi *et al.,* 2004; Bamba *et al*., 1993). Other uses include the treatment of abdominal and cervical pain, and of wounds as a local anti-septic agent (Bedi *et al.,* 2004; Bamba *et al.,* 1993). The ethanolic extract is used as an anti-fungal (Ngono *et al.*, 2006). The plant decoction is taken as a remedy for coughs and colds or in baths to treat skin diseases (Morton, 1981), also used in traditional medicine for wound healing and a local anti-septic agent (Inya-Agha *et al.,* 1987). The essential oil from *Chromolaena odorata* has been reported to exhibit insecticidal and antibacterial activities (Bouda *et al*., 2001). Despite their many uses and growing domestication at a local level, *Ceratotheca sesamoides* and *Chromolaena odorata* plants have remained predominantly underused and undervalued (Falusi *et al.,* 2002).

This present study investigated the extraction, partition, identification of the bioactive phytoconstituents, and determination of biological activities of the leaf extracts of *Ceratotheca sesamoides* and *Chromolaena odorata* against microorganisms.

2. MATERIALS AND METHODS

2.1. COLLECTION AND IDENTIFICATION OF PLANT SAMPLES

The fresh leaves of *Ceratotheca sesamoides* (Plate 1) and *Chromolaena odorata* (Plate 2) were collected from the wild at Government Residential Area and Bukan Ari in Lafia Local Government Area of Nasarawa State in Nigeria. The plant leaves were identified and authenticated by Prof. A.O. Ogaraku of the Department of Plant Science and Biotechnology of Nasarawa State University, Keffi.

Plate 1: Leaves and flower of C. sesamoides

Plate 2: Leaves and flowers of C. odorata

2.2. EXTRACTION AND FRACTIONATION OF THE LEAF EXTRACTS

Preparation of Samples

The fresh leaf samples of *Ceratotheca sesamoides* and *Chromolaena odorata* collected from the wild were washed thoroughly with running tap water to remove adhering dirt. Thereafter, the samples were rinsed with distilled water and air dried at room temperature. The dried leaves samples were manually ground into powder using mortar and pestle, sieved, weighed, packaged, and labeled for laboratory analysis.

Extraction of the Plant Samples

About 300 g each of the pulverized leaf samples of *Ceratotheca sesamoides* and *Chromolaena odorata* was packed into a thimble and placed inside a soxhlet extractor and extracted with 500 cm^3 methanol for 48 h. The resulting extract was then concentrated using a rotary evaporator (Büchi Rotavapor, R-205; Quickfit, England) and evaporated to dryness under vacuum.

Partitioning of Extracts

The crude methanolic extract was fractionated by modified Kupchan partitioning (Beckett and Stenlake, 1986) and Sabrina *et al*. (2016) method for solvent-solvent partition of crude extracts. The crude extract (35 g) from the mother solution, was partitioned using three solvents of different polarity in a separating funnel (n-hexane, chloroform and ethyl acetate). n-hexane (350 cm^3) was added to the crude extract and the funnel was shaken vigorously and then allowed to stand. The organic and aqueous phases were collected separately. This process was repeated three times and all the n-hexane fractions were collected together.

A similar procedure was repeated using chloroform and ethyl acetate, and their respective fractions were separately collected.

The residue fraction was preserved. All the fractions were collected separately and evaporated to dryness using rotary evaporator. Each fraction was subjected separately for phytochemical and antimicrobial analyses.

Phytochemical Screening of the Extracts

Phytochemical tests were used for the analysis of reducing sugar, tannins, saponins, cardiac glycosides, steroids, terpenoids ((Trease and Evans, 1983), flavonoids and alkaloids (Sofowora, 1982).

Microbial Test Organisms

The test organisms used for this study were *Klebsiella* spp., *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Candida albicans.* Their clinical isolates were obtained from the Innovative Biotechnology Limited, Keffi and were maintained at 4ºC in the refrigerator before use.

Bacterial Cultures Used

The bacteria and fungi cultures used in this study were obtained from the Innovative Biotechnology Limited, Keffi in Nasarawa State.

Antimicrobial Susceptibility Tests

The antimicrobial activity of the extracts against tested organisms using cup-plate agar diffusion method (Nair and Chanda, 2008). The Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were prepared, poured into petri dishes, allowed to solidify and labeled properly. Sterile cork borer (6 mm) was used; four holes were bored on the surface of the agar medium equidistant from one another and the base was sealed with sterile melted Mueller-Hinton agar 0.1 cm^3 of various concentrations of the extract 50 mg, 25 mg, 12.5 mg, 6.25 mg, 3.125 mg, 1.5 mg, and 0.75 mg were dispersed into the wells bore on MHA plates and SDA plates sealed with 10 CFU of test bacteria and *C. albicans* isolate. The MHA plates for test bacterial isolates were incubated at 37°C for 24 h whereas SDA plates for *C. albicans* were incubated at 37°C for 72 h. The extent of the radial growth was observed after incubation. The resulting zones of inhibition were measured with a millimeter ruler, surrounding bacterial growth. The experiment was conducted in duplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of solvent extract against test bacteria and fungi isolates were determined using agar diffusion method. The Mueller-Hinton agar (MHA) and Sabouraud dextrose agar plates containing different concentration of each extract (50 mg/cm^3, 25 mg/cm^3, 12.5 mg/cm^3, 6.25 mg/cm^3, 3.125 mg/cm^3, 1.56 mg/cm^3, 0.75 mg/cm^3) were prepared. 10 µl of the standardized test bacteria and fungi isolates were spotted on the surface of MHA plates and SDA plates, the plates were incubated at 37°C for 24 h for bacteria and 72 h for *C. albicans*. The minimum concentration of the extract that inhibits visible growth was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined using macro-broth dilution method. Different concentrations of solvent extracts were prepared using Mueller-Hinton and Sabouraud dextrose broth and 10 µl of standardized test bacteria was each inoculated into the 5 cm^3 tubes and shaken vigorously, and the tubes were incubated at 37°C for 24 h for bacteria isolates and 72 h for fungi isolates. 10µl from each test tube were spotted on extract free MHA plates and SDA plates and incubated at 37°C for 24 h for bacterial isolates and 72 h for *C. albicans*. The minimum concentration of the extracts that stop the growth of bacteria and *C. albicans* was recorded as the MBC.

3. RESULTS AND DISCUSSION

The results of the phytochemical analysis of the crude methanolic extract of *Cerathoteca sesamoides* (Table 1) revealed the presence of flavonoids in ethyl acetate, methanolic and residue extracts but absent in n-hexane and chloroform extracts. Saponins are present in chloroform, methanolic and residue extracts but absent in ethyl acetate and n-hexane extracts.

Table 1: Phytochemical Constituents of *Cerathoteca sesamoides* Leaf Extracts in Various Solvents

		Solvents				
S/N	Phytochemicals	n-hexane	Chloroform	Ethyl acetate	Methanol	Residue

1	Flavonoids	-	-	+	+	+
2	Saponins	-	+	-	+	+
3	Cardiac glycosides	+	+	+	+	+
4	Steroids	+	+	+	+	+
5	Terpenoides	+	+	+	+	+
6	Tannins	+	+	+	+	+
7	Alkaloids	-	-	+	+	+
8	Anthraquinones	-	-	-	-	-
9	Reducing sugar	-	-	-	-	-

+ = Present; − = Absent

Interestingly, the World Health Organization estimates that about 80% of the population living in Africa use traditional medicine for their primary health care. In addition, of the 300,000 plant species identified to be used in part or in whole for medicinal purposes, it is estimated that only 15% of them were studied to phytochemical level, 6% for their actual biological activities (Bothon *et al.,* 2014). The medicinal values of these plants lie in their component phytochemicals, which produce the definite physiological actions on human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Iwu, 2000). Tannins are present in n-hexane**,** chloroform, ethyl acetate, methanolic and residue extracts. Alkanoids are present in ethyl acetate, methanolic and residue extracts but absent in n-hexane and chloroform extracts. Steroids, terpenoids, tannins and cardiac glycosides are present in n-hexane**,** chloroform, ethyl acetate, methanolic and residue extracts. Alkanoids are present in ethyl acetate, methanolic and residue extracts but absent in n-hexane and chloroform extracts.

Thus, in the *C. odorata* sample, flavonoids, cardiac glycosides, steroids, terpenoids were present in all solvent extracts (Table 2). Tannins and alkaloids were present in all solvent extracts except n-hexane, while saponins were present in ethyl acetate, methanolic and residue extracts but absent in n-hexane and chloroform extracts. Anthraquinone and reducing sugars were absent in all extracts of *Chromoleana odorata* samples.

Table 2: Phytochemical Constituents of *Chromolaena odorata* Leaf Extracts in Various Solvents

		Solvents				
S/N	Phytochemicals	n-hexane	Chloroform	Ethyl acetate	Methanol	Residue
1	Flavonoids	+	+	+	+	+
2	Saponins	-	-	+	+	+
3	Cardiac glycosides	+	+	+	+	+
4	Steroids	+	+	+	+	+
5	Terpenoides	+	+	+	+	+
6	Tannins	-	+	+	+	+
7	Alkaloids	-	+	+	+	+
8	Anthraquinones	-	-	-	-	-
9	Reducing sugar	-	-	-	-	-

+ = Present; − = Absent

The results of the phytochemical analysis of the partition extracts of both *C. sesamoides* and *C. odorata* (Table 1 and Table.2) showed the varying proportions of the phytochemicals in the polarity of the solvents used; the polar bioactive constituents being extracted by the polar extracting solvents. *C. odorata* from Asia is a rich source of flavonoids (Pisutthanan *et al.,* 2006), but the bioactivity of these extracts has been little investigated. Flavonoids from flowers of *C. odorata* from Thailand, from which eight flavonoids were isolated and found to show weak to moderate anti-mycobacterial activity (Suksamrarn *et al.,* 2004). Environmental influences could lead to distinct phytochemical characteristics. Toyin *et al.* (2012) reported the presence of steroids, triterpenes, alkaloids, flavonoids, tannins, saponins, and glycosides in *C. sesamoides*. The presence of alkaloids, flavonoids and saponins in these plants is an indication that the plants can be used to cure dysentery and diarrhea as reported by Toyin *et al.* (2012).

Terpenes have anti-inflammatory properties, and have been reported to show excellent inhibition of soya lipoxygenase L-1 in *C. odorata* (Owoyele *et al.,* 2005; Bamba *et al*., 1993; Bedi *et al.,* 2004). Some of these bioactive

compounds have been associated with antibacterial activities (Nweze and Okafor, 2007), and may be identified for the medicinal activities of *C. sesamoides* and *C. odorata*. The presence of alkaloids and flavonoids in plant extracts is an indication of anti-inflammatory, antimalarial, and analgesic effects (Okwu and Josiah, 2006) that they could be used to treat malaria, diabetes, cancer, cardiac dysfunction, stomach ache, relief pains, and gastro intestinal disorder. 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). The presence of saponins suggests that *C. sesamoides* and *C. odorata* may contain useful phytoconstituents that could reduce blood cholesterol level and cancer risk due to its foaming ability that produces frothy effect (Okwu, 2003).

Tannins have been reported to have biological activities that may help in the treatment of many diseases (James *et al.,* 2007), as they prevent the development of microorganisms (Naveen *et al.,* 2008). Generally, tannin-containing plants such as *C. sesamoides* or *C. odorata* can be used to treat diarrhea, inflammations of mouth, sore throat and slightly injured skins (Naveen *et al.,* 2008). The plants can also be used to treat soft wounds, burn wounds and skin infections due to the presence of tannin and it may have astringent property (Osadebe and Ukwueze, 2004). The tannins in these plants may be responsible for their use in traditional medicine for wound healing and local antiseptic agents (Inya-Agha *et al.,* 1987), as the plants decoction are taken as a remedy for coughs and colds or in baths to treat skin diseases (Morton, 1981).

Table 3 and 4 shows the results of antimicrobial susceptibility of methanolic extracts of *C. sesamoides* and *C. odorata.* The results indicated the activity of extracts against all the tested clinical bacteria *Klebsiella* spp., *E. coli, P. aeruginosa, S. aureus,* but inactive against *C. albicans*. The Tables also indicated that *C. sesamoides* was more active than *C. odorata* for the range of concentrations tested. This finding agrees with the report of Gbadamosi and Egunyomi (2010) that the plants successfully inhibited some microbes. However, the report of Ngono *et al.* (2006) showed that the ethanolic extract of *C. odorata* is active against fungi. The activity of the methanolic extracts against *P. aeruginosa* (at 12.5 mg/ml) in both *C. sesamoides* and *C. odorata* showed that the plants can treat burns, wound swab, soft tissue infection and skin infections. The methanolic extract of these plants showed highest activity against *S. aureus* (at 6.25 mg/ml). This is in agreement with the findings of Bouda *et al.* (2011), who reported that essential oils from *C. odorata* exhibit insecticidal and antibacterial activities. Irobi (1997) reported activity of lower concentrations of crude ethanolic extract of leaves of *C. odorata* against *P. aeruginosa* (8.0 mg/ml), *Streptococcus faecalis* (6.0 mg/ml) and *S. aureus.*

Table 3: Antimicrobial activity of Methanolic extract of *Cerathoteca sesamoides*

		Concentration of Methanolic Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	16.0 ± 0.71	12.0 ± 2.12	0	0	0	0	0
2	Klebsiella spp.	20.0 ± 0.71	17.0 ± 2.12	9.0 ± 2.12	0	0	0	0
3	P. aeruginosa	19.0 ± 2.82	13.0 ± 0.71	9.0 ± 0.71	0	0	0	0
4	S. aureus	24.0 ± 1.41	19.0 ± 1.41	16.0 ± 1.41	11. 0 ± 0.71	0	0	0
5	Candida albicans	0	0	0	0	0	0	0

Table 4: Antimicrobial activity of Methanolic extract of *Chromolaena odorata*

		Concentration of Methanolic Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	23.0 ± 1.41	18.0 ± 0.21	0	0	0	0	0
2	Klebsiella spp.	22.0 ± 0.71	17.0 ± 1.41	9.0 ± 1.41	0	0	0	0
3	P. aeruginosa	20.0 ± 1.41	15.0 ± 0.71	9.0 ± 1.41	0	0	0	0
4	S. aureus	29.0 ± 1.41	23.0 ± 0.71	17.0 ± 0.71	12.0 ± 0.71	0	0	0
5	Candida albicans	0	0	0	0	0	0	0

Table 5 and 6 showed the results of antimicrobial susceptibility of chloroform extracts of *C. sesamoides* and *C. odorata.* The results indicated that the chloroform extracts of both plants had activity against all the tested clinical bacteria *Klebsiella* spp., *E. coli, P. aeruginosa, S. aureus,* and *C. albicans*.

Table 5: Antimicrobial activity of chloroform extract of *Cerathoteca sesamoides*

		Concentration of Chloroform Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	20.0 ± 1.41	14.0 ± 1.41	12.0 ± 0.71	0	0	0	0
2	Klebsiella spp.	21.0 ± 0.71	17.0 ± 1.41	13.0 ± 0.71	9.0 ± 0.71	0	0	0
3	P. aeruginosa	19.0 ± 1.41	17.0 ± 1.41	12.0 ± 0.71	9.0 ± 0.71	0	0	0
4	S. aureus	20.0 ± 2.82	15.0 ± 1.41	9.0 ± 1.41	0	0	0	0
5	Candida albicans	13.0 ± 0.49	9.0 ± 5.52	0	0	0	0	0

Table 6: Antimicrobial activity of chloroform extract of *Chromolaena odorata*

		Concentration of Chloroform Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	23.0 ± 1.41	18.0 ± 0.71	10.0 ± 2.12	0	0	0	0
2	Klebsiella spp.	25.0 ± 1.41	19.0 ± 1.41	15.0 ± 1.41	9.0 ± 1.41	0	0	0
3	P. aeruginosa	21.0 ± 0.71	17.0 ± 2.82	12.0 ± 2.82	9.0 ± 2.12	0	0	0
4	S. aureus	21.0 ± 1.41	16.0 ± 1.90	10.0 ± 0.00	0	0	0	0
5	Candida albicans	14.0 ± 0.36	9.0 ± 2.12	0	0	0	0	0

The chloroform extract inhibited the growth of *Klebsiella* spp. at 21 mm by *C. sesamoides* and at 25 mm by *C. odorata,* which indicated the use of these plants in treatment of wound infections, burns, urinary tract infections and chronic liver diseases.

The ethyl acetate extracts of both *C. sesamoides* and *C. odorata* were active against the bacteria *Klebsiella* spp., *E. coli, P. aeruginosa, S. aureus,* and *C. albicans*; but *C. odorata* was more active against the micro-organisms (Table 7 and Table 8). The activity of ethyl acetate extracts (at 12.5 mg/ml) against *E. coli* in both *C. sesamoides* and *C. odorata* indicated that the plant leaves can be used to treat infections due to these causative agents, such as urinary tract infections. The activities against *C. albicans* also indicated that the plant extracts could be effective at treating candidiasis.

Table 7: Antimicrobial activity of Ethyl Acetate extract of *Cerathoteca sesamoides*

		Concentration of Ethyl acetate Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	20.0 ± 0.71	12.0 ± 2.12	9.0 ± 0.71	0	0	0	0
2	Klebsiella spp.	17.0 ± 0.71	10.0 ± 0.71	0	0	0	0	0
3	P. aeruginosa	16.0 ± 1.41	9.0 ± 2.12	0	0	0	0	0
4	S. aureus	18.0 ± 2.82	13.0 ± 1.41	8.0 ± 1.41	0	0	0	0
5	Candida albicans	19.0 ± 0.71	12.0 ± 0.71	0	0	0	0	0

Table 8: Antimicrobial activity of Ethyl Acetate extract of *Chromolaena odorata*

		Concentration of Ethyl acetate Extract (mg/ml)/zone of inhibition (mm)						
S/N	Test Organism	50	25	12.5	6.25	3.125	1.56	0.78
1	Escherichia coli	22.0 ± 0.71	15.0 ± 0.71	9.0 ± 1.141	0	0	0	0
2	Klebsiella spp.	19.0 ± 0.71	12.0 ± 0.71	0	0	0	0	0
3	P. aeruginosa	16.0 ± 0.71	9.0 ± 0.71	0	0	0	0	0
4	S. aureus	18.0 ± 0.71	13.0 ± 0.71	9.0 ± 0.70	0	0	0	0
5	Candida albicans	17.0 ± 1.41	11.0 ± 0.47	0	0	0	0	0

The minimum inhibition concentration (MIC) is the lowest concentration of an antibacterial agent necessary to inhibit visible growth or bacteriostatic. MICs are used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentrations of antibiotic/antiseptic over a defined period in terms of inhibition of microbial population growth. These evaluations can be quite useful during the R&D phase of a product to determine appropriate concentrations required in the final product, as the concentration of drug required to

produce the effect is normally several hundred to thousands of times less than the concentration found in the finished dosage form. The minimum inhibitory concentration of the extracts was determined to ascertain the potency of the extracts against the test organisms at different concentrations in which the growth of all the test organisms were inhibited at concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.5 mg/ml with the exception of *Candida albicans* which shows only growth at the concentrations of 50 mg/ml and 25 mg/ml in a chloroform solvent extracts as shown in Tables 3, 4, 5, 6, 7, 8 and 10.

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill ≥99.9% of a particular bacterium (or, bacteriocidal). In this present study, the lowest MBC for *Cerethoteca sesamoides* and *Chloromolaena odorata* was observed for *E. coli, Klebsiella* spp. and *Pseudomonas aeruginosa* at concentration of 50.0 mg/ml each respectively from chloroform extracts. However, a 50 mg/ml MBC was recorded for *Staphylococcus aureus* for the methanolic extracts of these plants as presented in Table 9. Tables 9 and 10 showed that while the MIC ranged between 12.5 mg and >50 mg, the MBC ranged between 50 mg and >50 mg. Tripathi (2013) reported that the closer the MIC is to the MBC, the more bactericidal the compound or extract.

Table 9: Minimum bactericidal concentration (mg/ml) of extracts against bacteria and fungi isolates for *Cerathoteca sesamoides* and *Chromolaena odorata*

	E. coli		Klebsiella Spp		P. aeruginosa		S. aureus		C. albicans	
Solvent Extracts	C.S	C.O	C.S	C.O	C.S	C.O	C.S	C.O	C.S	C.O
Chloroform	50.0	50.0	50.0	50.0	50.0	50.0	50.0	≥50.0	≥50.0	≥50.0
Ethyl acetate	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0
Methanolic	≥50.0	≥50.0	50.0	≥50.0	50.0	≥50.0	50.0	50.0	50.0	≥50.0

C.S = *Cerathoteca sesamoides* ; C.O = *Chromolaena odorata*

Table 10: Minimum inhibitory concentration (mg/ml) of extracts against bacteria and fungi isolates for *Cerathoteca sesamoides* and *Chromolaena odorata*

	E. coli		Klebsiella Spp		P. aeruginosa		S. aureus		C. albicans	
Solvent Extracts	C.S	C.O	C.S	C.O	C.S	C.O	C.S	C.O	C.S	C.O
Chloroform	50.0	25.0	25.0	12.5	50.0	50.0	≥50.0	25.0	≥50.0	≥50.0
Ethyl acetate	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0	50.0	≥50.0
Methanolic	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0	≥50.0

C.S = *Cerathoteca sesamoides* ; C.O = *Chromolaena odorata*

4. CONCLUSION

The presence of bioactive constituents (including phytochemicals) have made *Cerathoteca sesamoides* and *Chromolaena odorata* very useful in traditional medicine. Among the four different solvents used in partitioning methanolic and ethyl acetate extracts of both plants contain flavonoids, tannins, alkaloids, terpenoids, saponins, steroids, and cardiac glycosides. Saponins were absent in the chloroform extract of *C. odorata* and the ethyl acetate extract of *C. sesamoide*. While flavonoids were present in the n-hexane extracts of *C. odorata*, they were absent in *C. sesamoides*. Anthraquinone and reducing sugar were absent in all the solvent extracts of both medicinal plants. This shows that the presence of these bioactive components is related to the type and polarity of the extracting solvents. The antimicrobial susceptibility tests showed that n-hexane and residue extracts of both plants had no activity against the tested microorganisms. The chloroform and ethyl acetate extracts of *C. sesamoides* and *C. odorata* were active against all the tested clinical bacteria *K.* spp., *E. coli, P. aeruginosa, S. aureus,* and *C. albicans* at high concentrations. The methanolic extracts of both plants were active against the bacterial isolates but inactive against the fungus *C. albicans*. The minimum bactericidal concentrations of these plant extracts ranged between 50 mg and >50 mg, while the minimum inhibition concentrations ranged between 12.5 mg and >50 mg. Consequently, the chloroform or ethyl acetate extracts of the leaves of these plant drugs could be used to treat infections due to these causative agents, such as urinary tract infections and candidiasis.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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