International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 3 Sept, 2025 Research Article

Phytochemcial Screening and Antimicrobial Activity of Pasture Weed (*Cyathula prostrata* Linn) on Selected Fungi

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ABSTRACT

The drive in search of antimicrobial potential of plant extracts (phytochemicals) has increased in recent years. This has inspired the investigation on some indigenous medicinal in traditional medicine. The antimicrobial effect of aqueous leaf extract of Pasture weed (*Cyathula prostrate*) was tested on fungal isolates of *Penicillium chrysogenum, Fusarium oxysporum, Aspergillus niger* using the Agar well diffusion method at different extract concentration of 20 ml, 10 ml, 5 ml and 2.5 ml. The conventional antifungal agent Nystatin was used at the same or respective concentrations as positive control standard to determine the sensitivity of the competition. Results obtained showed that all the test isolates were inhibited at varying degrees of the leaf extract concentration. The antimicrobial activities of the plant leaf extract competed with the antibiotics significantly at $P<0.05$. The greatest activities aqueous leaf extracts at 20ml concentration which infected mycelial growth of fungal isolates ranged from 19.50mm $\pm$ 0.5 in *A. niger* to 23.50mm + 0.50 in *P. chrysogenum*. It was found that the aqueous leaf extracts of the plant *C. prostrate* possess a broad-spectrum of antimicrobial properties against the test organisms. The aqueous leaf extracts of *C. prostrata* was screened for bioactive components and the observed phytochemicals were Alkaloids, saponins, flavonoids, terpenoids, steroids and cardiac glycosides. This findings therefore, that suggest further studies to be carried out on the market of plant extracts in order to isolate, identify so as to maximize the medicinal potential of the plant. These findings highlights the need for further studies to be carried out on the phytochemical components of the leaf extract of *C. prostrata* that possess antifungal activity so as to maximize the medicinal potentials of the plant.

Keywords: Phytochemical screening, antifungal, medicinal plants, aqueous leaf extract and *cyathula prostrata.*

Introduction

Cyathula prostrata (L) *Blume (Amaratheceae*) is a herb and a medicinal plant that is used in Tropical Africa, Asia and Australia to treat many ailments; rheumatic fever, dysentery, wounds and eye problem (Burkhill, 1985). Plants and their extracts since time immemorial have been applied as herbal remedies for diverse human ailments and various microbial diseases. Presently, plant is still being utilized by numerous developing countries as sources of therapeutic agents because they believe medicinal plants are readily available, accessible, affordable, potent, and with relatively lower incidences of adverse reactions compared to modern conventional drugs (Adomi, 2008). Base on the growing knowledge of potency of traditional medicinal plants coupled with fact that numerous infections agents are becoming resistant to synthetic drugs, researchers all over the World have intensified the screening of these acclaimed medicinal plants in order to provide a documented scientific backing and ultimately recommend them as novel sources of future antimicrobial agents (Proph and Onoagbe, 2012).

Traditionally, various preparations of the leaves, stems and roots of this plant are used to treat a range of illnesses including articular rheumatism, cough, skin diseases, scabies, craw-craw, snake bites, bruises, liver problem, dysentery, diarrhoea, nausea, cholera vomiting blood, and many others in Nigeria and other African countries (Odugbemi 2006, Kannappan 2009).

Previous studies have shown that the secondary metabolites or phytochemicals present in plants help to protect against external stresses such as pests and diseases (Mazid , *et al,* 2011).

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Currently, ethno medicinal plants are being screened with a view to identifying those with potent secondary metabolites for the synthesis of antimicrobial agents against pathogens are currently posing a great challenge to global food security and sustainability (Al-Samarrai *et al.,* 2012). However, natural products from higher plants continue to be used in pharmaceutical either pure or as extracts (Gogtay *et al.,* 2002). Despite the availability of different approaches for the discovery of therapeutic agents, natural products still remain as one of the best reservoirs of new structural types (Hostettmann, 1999).To constant effort to improve the efficacy and ethics of modern medicinal practice, researchers are increasingly turning their attention to folk medicine as a source of new drugs (Wayne, 1998; Hoareau and Dasilva, 1999).

Nonetheless, only a few medicinal plants have been validated for their medicinal virtues, moreover, the biological activities of such medicinal plants against certain human pathogenic bacteria and fungi are poorly investigated (Gurib-Fakim *et al.,* 1993, 1996a, b). Also, the emergence of resistant pathogens to many of the commonly used antibiotics has provided an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance to currently available antimicrobial agents (Balandrin *et al.,* 1985; Xu and Lee, 2001). Plant-based antimicrobials represent a vast untapped source for medicines and hence have enormous therapeutic potential (Phillipson, 1994). They are effective in the treatment of infections while mitigating many of the side effects associated with synthetic antimicrobial (Mathews *et al.,* 1999; Bagghi, 2000)

Despite the arrays of traditional applications to which the leaf, stem and root of *Cyathula prostrata* are subjected to, available literature revealed that there are paucity of information on the scientific elucidation of this plant as remedy for the acclaimed. This study was carried out to investigate the phytochemical content and antimicrobial activity of *C. prostrata* on selected fungi. The specific objectives of this research are to; evaluate the phytochemical constituent of *C. prostrata* leaves, determine the antifungal activity of aqueous and ethanolic leaf extracts of *C. prostrata. C. prostrata* is an erect or decombant plant with 20 cm to 60 cm high, sometimes more. The angular stem is covered with erect hairs.

The opposite leaves, 4 to 9 cm long, are short-stalked, tinted red below and at the edge, especially when young. The leaf blade is elliptic, with the top tapered to a tip. The flowers are small and greenish, with less than 3 mm long. They are arranged in long terminal and lateral spikes. The fruit hidden in a ball of small hooked hairs are headed downward to maturity.

It is more or less erect or decumbent. The plant is branched as from the base. The plant has a taproot system. The stem is quadrangular, full; it is sometimes almost cylindrical and longitudinally striated. It is pubescent with erect hairs or nearly glabrous. The leaves are simple and opposite, sometimes tinged with purple beneath and at the edge, especially when young. The petiole is 5 to 15 mm long and pubescent. The leaf blade is elliptic, obviate, often with a diamond shape for the big leaves. It measures 1.5 to 8 cm long and 1 to 4.5 cm wide, wedged base, acute or acuminate apex. The margin is entire. 5-6 pairs of parallel curved lateral veins. Both sides are pubescent to sub-glabrous in older leaves. The inflorescence is arranged in terminal spikes of up to 35 cm long, or lateral at the axils of the upper leaves, but smaller in size. The rachis is pubescent. The flowers are grouped in small dense glomerules at the top, spaced quickly towards the base. These glomerules are first erect towards the top then flips over and apply to the axis of the spike after flowering.

The floral glomerules are subglobose, shortly stalked, with basically a small, apiculate, oval bract at the base. They are composed of 2 to 3 fertile ovoid flowers of 2 to 3 mm, subtended by oval pubescent bracts, mucronate at the top. The tepals are 2 to 3 mm long, elliptical oblong, trinervate. The lateral flowers are placed between two small sterile accrescente flowers which are transformed into bracts (7 to 8 per flower). These sterile flowers are reduced to crooked edges assembled at the base on a pedicel of 1 mm. They are long-acuminate, acute of 1 to 2 mm, curved hook. The ovary has only one ovule. It is more or less obovoid, and 0.7 mm long. The style is slender, with a rounded stigma. The fruit is an ovoid capsule, very thin with almost membranous pericarp, containing only one seed. It is surmounted on style. The seed is lenticular ovoid, 1.5 mm long, brown, shiny and smooth. *Cyathula prostrata i*s native to Africa, Asia and Papua New Guinea, Palau, Timor-Leste and North East Australia in Oceania.

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It now has a pantropical distribution after being introduced in other countries and Oceanic and Central and South America.

The aerial parts in decoction are drunk against cough, and a decoction of the roots is used against dysentery (Djab et al., 2017). As a plaster, it is used for caterpillar itch, around the neck for cough and on the belly for intestinal worms or shingles (PROTA, 2015). In Indonesia, the leaves mashed with water are a remedy for cholera, and an infusion of the whole plant is taken for fever and dysentery. In Papua New Guinea, the juice of the stem is used as an abortifacient. In Sierra Leone, the roots are used for this purpose. In the Philippines and Guinea, the ash of the burnt plant mixed with water is rubbed on the body for scabies and other skin ailments. In Thailand, the stem in decoction is taken as a diuretic and to increase menstrual discharge (Djab et al., 2017); the leaves are used for irritations of the throat; the flowers as an expectorant; and the roots against abnormal and frequent urination. In Vietnam, the roots in decoction are commonly drunk for colds and cough (Djab et al., 2017). In Indo-China, the same preparation is used for rheumatism and dropsy (Djab et al., 2017). In China, the stem and leaves are used as a mild laxative. In Taiwan, a decoction of the leaves is applied to snakebites. Throughout Africa, the plant is used to treat dysentery. In Cameroon, the plant is prescribed for articular rheumatism. In Cote d'Ivoire, the sap of the plant is used as ear drops for otitis and for headache and the pulped plant is used on sores, burns and fractures as a *haemostatic* and *cicatrizant.* In Gabon and Congo (Brazzaville), the leaves are eaten as a vegetable" (PROTA, 2015). *Cyathula prostrata* plant is often harvested from the wild for local use, especially for medicinal purposes but also as a food. According to *Djah et al. (*2017) the leaves are typically used for treatment and management against rheumatic fever, dysentery, wounds and eye trouble, the sap is traditionally used as an ear drop to treat otitis and is also applied to skin and burns. The leaves, mashed with water are used as a remedy for treatment of cholera disease. A decoction of the leaves is applied to snakebites. The juice from macerated leaves is applied to cuts and bruises as an antiseptic and the macerated leaves themselves are applied to wounds to stop bleeding. The decoction of the leaves is used to ease irritations of the throat. The stem and leaves decoction is administered as a mild laxative. The juice extracted from the stem is used as an abortifacient.

The aim of this study therefore, is to carry out the phytochemcial screening of pasture weed (*Cyathula prostrata* Linn) and the effect of its and antimicrobial activity on selected fungi so as to maximize the medicinal potentials of the plant.

Materials and Methods

Collection of Plant material and Identification

Fresh plants of *Cyathula prostrata* were collected from farmland around the Rivers State University Teaching and Research Farm of Rivers State University, Nigeria and was identified by Prof. B .O Green, a plant taxonomist in the Department of Plant science and Biotechnology of the Rivers State University, Port Harcourt, Nigeria.

Sterilization of Materials

All glasswares used in this research were washed with detergent, rinsed with distilled water, air dried and sterilized on a hot air oven at 121^0C for 2 hours. Each of the materials was wrapped with aluminium foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121^0C for 15 minutes. Cork borers and glass rods were sterilized by dipping into 70% alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 75% alcohol before and after each experiment (Chukunda *et al.,* 2019).

Source of Test Micro- Organisms

The stock culture of the microbial isolates of *Penicillium chrysogenum, Fusarium oxysporum,* and *Aspergillus niger* (fungi) were collected from the Department of Forestry and Environment Laboratory Mycology of the Rivers State University, Port Harcourt, Nigeria. The cultures were subcultured and maintained at 35°C on sabouraud dextrose agar used for further study (Chukunda *et al.,* 2019).

Standardization of Test Organisms

Prior to antimicrobial sensitivity test, 0.2ml of overnight culture of each organism was dispensed into 20ml of sterile Mueller Hinton Broth (Hi-Media, India) and then incubated for about 16-24h to standardize the cultures to approximately 10^6cfu/ml (Collins *et al* 1995).

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Preparation of Plant Materials/ Extraction

The fresh leaves of the plant were washed thoroughly and air dried for two weeks before grinding. Aqueous extraction was used. Then 150g of the grinded leaf was weighed and put in 375ml of distilled water and allowed to stand for 48 hrs, agitated or shaken for 45 mins. The extract was filtered using British Standard Mesh filter and concentrated by using air drying under constant air current and water bath at 50^0C. The extract was then transferred into a clean container and stored in the refrigerator at -4^0C until required for use while Soxhlet method was carried out by continuously extracting the grinded plant leaf with an organic solvent (ethanol) for 4-6 hours.

Phytochemical Screening of Extract

The extracts of *Cyathula prostrata* was analysed quantitatively and qualitatively to determine the presence of some phytochemicals such as steroids, saponins, alkaloids, flavonoids, terpenoids, cardiac glycosides, and tannins using the standard procedures previously reported (Grease *et al* 1996).

Qualitative Phytochemical Analysis

Test for Alkaloids

1ml of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent or Wagner's reagent (solution of iodine in Potassium iodide) or Dragendorff's reagent. The formation of a cream colour with Mayer's reagent and reddish-brown precipitate with Wagner's and Dragendorff's reagent give a positive test for alkaloids (Hikino *et al.,* 1984).

Test for Flavonoids

Three milliliter (3ml) of 1% Aluminium chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution was added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicates a positive test for flavonoids (Sofowara, 1993; Harborne, 1973).

Test for Saponins

Five ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filterate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirms a positive presence of Saponin (Ejikeme *et al.,* 2014).

Test for Taninns

One ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue – black coloration which confirms the presence of tannin (Ejikeme *et al.,* 2014).

Test for Steroids

Two ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H_2SO_4. The colour changed from violet to blue or green indicated the presence of steroids (Ejikeme *et al.,* 2014).

Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed with 2ml of chloroform, and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids (Ejikeme *et al.,* 2014).

Test for Cardiac Glycosides and Cardenolides (Keller – Killani test)

Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates deoxysugar characteristics of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicated the positive presence of glycoside (Hikino *et al.,* 1984).

Antimicrobial Assay

The agar well diffusion method by (Collins *et al.,* 1995) with slight modification, was adopted for this assay.

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Mueller Hinton Broth (Hi-Media, India) was prepared as specified by the manufacturer, autoclaved and poured ascetically into sterile Petri dishes and allowed to gel. The microorganisms used were *Penicillium chrysogenum, Fusarium oxysporam* and *Aspergillus niger*. Then a loopful of the standardized fungal spore suspension (10^6cfu/ml) was streaked evenly on each gelled agar plate. Aqueous leaf extracts and water was used in the serial dilution of the same concentration. 50µl extract was inoculated into four wells (6 mm Diameters) earlier bored with a sterile cork borer in each plate. The positive control was Nystatin for fungal. The plates were allowed to stand for 30minutes on the work bench for pre-diffusion of the extract to proceed before the growth of the organism commenced. The plates were incubated at 37°C for 24h. The whole experiment was carried out in triplicate and the antimicrobial activity of the extract was determined after incubation period by measurement of mean diameter zones of inhibition produced by the extract against the test organisms and results were recorded in millimeters(mm) using a transparent ruler (Chukunda *et al.,* 2020).

Experimental Design/ Statistical Analysis

The experiment was laid out in a completely randomized design; the treatments were replicated three times. Data obtained were subjected to one way analysis of variance (ANOVA). The mean separation was done using Duncan Multiple Range Test (DMRT) at the probability of 5% (P<0.05).

Results

The phytochemical screening of aqueous and ethanolic extract of *Cyathula prostrata* leaves revealed the presence of some important bioactive components as shown in Table 1. Both aqueous and ethanolic extracts of *cyathula prostrata* contains very high amount of Saponin (+++), alkaloids (++), flavonoid (++), and cardial- glycosides (++) were present in moderately high amount in the both extracts. Terpenoids and steroids were present in trace amounts, Tanins (-) were not detected in either of the extracts.

Table 1: Phytochemical Properties of Leaf of *Cyathula prostrata*

Phytochemicals	Extract of *Cyathula prostrata*	
	Ethanol Extract	Aqueous Extract
Alkaloids	++	++
Flavonoids	++	++
Saponins	+++	+++
Tanins	-	-
Terpenoids	+	+
Steroids	+	+
Cardiac glycosides	++	++

Key: Heavily present +++; moderately present, ++ present in trace amount +, absent: -

Results of the antifungal activity of aqueous extracts of *Cyathula prostrata* on some selected fungal isolates are presented in Table 2 and 3**.** The results on aqueous extract of *C. prostrata* inhibited the growth of fungal. However, the extracts competed significantly ($P \leq 0.05$) with synthetic antibiotics which served as control. The result revealed that the aqueous plant extract of *Cyathula prostrata* significantly inhibited the mycelial growth of the test fungi, *Penicillum chrysogenum, Fusarium oxysporum* and *Aspergillus niger.* Results indicates that the extracts were effective though at varying degrees of concentration at 20ml *P. chrysogenum* (22.50mm±0.50) had the highest zone of inhibition followed by *A. niger* (20.50mm±0.50) and *F. oxysporum* (19.50mm±0.50) had the least zone of inhibition.

Table 2 Antimicrobial activity of aqueous leaf extracts of *C. prostrata* on fungal isolates

Extract Conc (ml)	*Penicillim chrysogenum*		*Fusarium oxysporum*		*Aspergillus niger*	
	Extract	Antibiotics (Nystatin)	Extract	Antibiotics (Nystatin)	Extracts	Antibiotics (Nystatin)
20	22.50± 0.50 ^{a}	26.50 ± 0.50 ^{a}	19.50 ± 0.50 ^{b}	24.50 ± 0.50 ^{a}	20.50 ± 0.50 ^{a}	23.50 ± 0.50 ^{a}
10	16.50 ± 0.50 ^{b}	23.50 ± 0.50 ^{a}	14.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{b}	17.50 ± 0.50 ^{b}	21.50 ± 0.50 ^{a}
5	12.50 ± 0.50 ^{b}	19.50 ± 0.50 ^{b}	10.50 ± 0.50 ^{c}	14. 50 ± 0.50 ^{c}	13.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{b}
2.5	9.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{b}	8.50 ± 0.50 ^{c}	12.50 ± 0.50 ^{c}	10.50 ± 0.50 ^{c}	13.50 ± 0.50 ^{c}

Key: Mean values with the same superscripts (a, b, c) in the column are not significantly ($p \leq 0.05$) different by DMRT

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Table 3: Antimicrobial Activity of Ethanol leaf extracts of *Cyathula prostrata* on fungal

Extract Conc (ml)	*P. chrysogenum*		*F. oxysporum*		*A. niger*	
	Extract	Antibiotics (Nystatin)	Extract	Antibiotics (Nystatin)	Extracts	Antibiotics (Nystatin)
20	17.50± 0.50 ^{b}	24.50 ± 0.50 ^{a}	15.50 ± 0.50 ^{b}	22.50 ± 0.50 ^{a}	15.50 ± 0.50 ^{b}	23.50 ± 0.50 ^{a}
10	14.50 ± 0.50 ^{c}	20.50 ± 0.50 ^{b}	12.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{c}	13.50 ± 0.50 ^{c}	19.50 ± 0.50 ^{b}
5	10.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{c}	8.50 ± 0.50 ^{c}	13. 50 ± 0.50 ^{c}	10.50 ± 0.50 ^{c}	16.50 ± 0.50 ^{b}
2.5	7.50± 0.50 ^{c}	11.50 ± 0.50 ^{c}	6.50 ± 0.50 ^{c}	9.50 ± 0.50 ^{c}	18.50 ± 0.50 ^{c}	12.50 ± 0.50 ^{c}

Key: Mean values with the same superscripts (a, b, c) in the column are not significantly ($p \leq 0.05$) different by DMRT

Discussion

Generally, the effectiveness of any antimicrobial compound depends on the ability of the microbes to inhibit or stop the growth of any micro-organism. Because of the high genetic variability of micro-organisms, they seem to however develop the ability to rapidly evade the action of antimicrobials by becoming resistant to them. It becomes necessary therefore to consistently look for newer means of eliminating microbial threat causing infection. Results of the antimicrobial activity of *Cyathula prostrata* against fungal at different concentration in Tables 2 and 3; indicate that the extracts are effective though at varying degree. This result is in agreement with Enabulele *et al.,* (2012) and Karou *et al.,* (2006) which reported that susceptibility of bacteria to plant extracts on the basis of zones of inhibition varies according to strains and species.

The differences observed in the antimicrobial activities assays suggest the susceptibility of the test microorganisms to the various secondary metabolites present in the crude leaf extract of *C. prostrata.* The composition of these secondary metabolites in turn varies from species to species, climate conditions and physiological state of developments of the plants (Hussain & Deeni, 1991).

Results from the current study show that ethanotic leaf extracts of *C. prostrata* gave poor inhibition zones on both fungi isolates. (Cragg *et al.,* 1994) reported that there are great difference between the activity of aqueous and organic extracts in their anti- Hiv screening: about 34% of the plant was active in aqueous extract and only 4% in organic extract.

This observation disagree with the works of Awe *et al.,* (2009) and Anibijuwon *et al.* (2010) who reported that the ehanolic extract of *Piliostgma reticulatum* and *Aspila africana* generally displayed the greatest activities, followed by the hot aqueous and cold aqueous extracts against *S. aureus, S. faecalis, S. typhimunum, P. aeruginosa, K. pneumonia and S. dysentriae* The results of the aqueous leaf extract of *C. prostrata* on the selected fungi agrees Chukunda *et al.* (2020), who reported that aqueous extracts of *Enantia chlorantia* bark inhibited the fungal spoilage of avocado pear. Traditionally, plant parts are soaked in water and alcohol based solvents for days before they are administered. The result from this study therefore, lends credence to the traditional use of these solvents to obtain plant extracts which were found to be highly effective against all the test organisms. Results from this study shows that the aqueous and ethanolic leaf extracts of *C. prostrata* possesses more of anti-fungal agent than anti- bacteria. This may be attributed to structural differences between prokanyotic bacteria and eukaryotic fungal agents, anti-microbial agents should bind to sterols in eukaryotic membrane so as to exhibit their action, whereas this step is not needed for bacterial cells (Medoff and Kayashi 1993).

Comparison of the percentage of effectiveness of the various extracts at different concentrations with the control antibiotic indicates that at the concentration they were used, most of the extract were more than half as effective as the control even with the fact that they were in their crude state and not as pure as the control antibiotic . The fact too that the extracts showed a broad spectrum of activity is significant in the drive to developing therapeutic substances against multi-drug resistant organisms.

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Conclusion

In conclusion, this study has demonstrated that the leaf extracts of *Cyathula prostrata* possess bioactive ingredient with in vitro antifungal activities against some phyto-pathogens, thereby justifying the application of their extracts as traditional herbal medicine. It is worth noting that in vitro finding is not always dependable because plants which are effective in vitro might not work when used in vitro and some plants which showed little or no effect in vitro study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in animal bodies Also, identification of the active constituents is needed to exploit them in evaluating their efficacy and safety field trials against the test pathogens. Based on the findings in this research work the following can be recommended that extracts *C. prostrata* can be used as an alternative to synthetic fungicides. Aqueous extracts can be preferably used in anti-microbial studies since it is effective, cheap and eco-friendly.

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Citation: Chukunda *et al.* (2025). Phytochemcial screening and antimicrobial activity of pasture weed (*Cyathula prostrata* linn) on selected fungi. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 243 – 250.