Research Article

Qualitative analysis of phytochemicals, antibacterial activity, and mycelial growth inhibition of three different plants

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ABSTRACT

Objective: The objective of this study was to screen the phytochemical compounds, antibacterial activity, and mycelial growth inhibition of three different medicinal plants, namely *Couroupita guianensis*, *Morinda tinctoria,* and *Tabernaemontana divaricata*. **Materials and Methods:** The antibacterial activity of methanolic leaf extracts was determined by well diffusion method. Antifungal activity was determined by seeded agar technique and its phytochemical screening was also performed. **Results:** Among three plants, *C. guianensis* methanolic leaf extract shows better antibacterial activity. In mycelial growth inhibition, all three plants possess complete mycelial growth inhibition against some tested fungi, but *C. guianensis* leaf extract possesses better inhibition against all tested fungi. **Conclusion:** Among three different plants, *C. guianensis* methanolic leaf extract exhibited better antimicrobial activity and this may be due to the presence of phenolic compounds, tannin, and terpenoids.

KEY WORDS: Antimicrobial activity, Couroupita guianensis, Morinda tinctoria, Phytochemicals, Tabernaemontana divaricata

INTRODUCTION

Our nature has enriched with botanical wealth and a large number of diverse types of plants. Plants are an important source of medicines since ancient times and 70% of the worldwide population still relies on traditional plant-based medicine[1] for primary health care. Plant-based medicine is used for its better cultural acceptability, better compatibility with the human body, and fewer side effects.[2]

The increase use of commercial antimicrobial drugs leads to the microbial resistance against antibiotics[3] and threatens public health by reducing the effectiveness of treatments and increases morbidity, mortality, and health-care costs.[4] In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression, and allergic reactions.[5] To overcome this situation, a new and effective therapeutic agent has to be developed from medicinal plants.[6]

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According to the World Health Organization,[7] medicinal plants would be the best source to obtain a variety of drugs, and it supports the use of traditional medicine and it was proven to be efficacious and safe.

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[8] The antimicrobial activity of different herbal extracts in different regions of the world has been reported.[9]

Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.[10] The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate, and phenolic compounds. The beneficial effects of medicinal plant materials are due to the presence of secondary products or phytochemicals present in the plants, which are synthesized and deposited in specific parts or all parts of the plant.[11] These actions of medicinal plants are unique to a particular plant species or group.

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Antimicrobial screening of plant extracts and phytochemicals is the starting point for antimicrobial drug discovery.[12] Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry.[13] The effect of plant extracts on microorganisms has been studied by large number of researchers in different parts of the world.[14]

Almost all parts of these three plants were used in the treatment of various ailments. The leaves of the plants possess antibiotic, antiseptic, and analgesic qualities[15] used to cure skin diseases and malaria. It possesses herbal hand wash formulation and yielded an aliphatic triterpene. Leaves are useful as tonic, febrifuge. It is also used for curing dyspepsia, diarrhea, ulceration, stomatitis, and digestion and used to prevent the formation and proliferation of tumors including malignant ones.[16] It is reported to have hypotensive and immune-enhancing effects. The leaves of the medicinal plants have the active constituents, namely isatin, which has cytotoxic activity against cancer cell lines and coronaridine shows promise as a potential treatment for opiate and cocaine addiction.

Other parts of the plants can be used as wound disinfectants, cure toothache, and to boost the immune system. It is also used in the preparations to cure gastritis, scabies, bleeding piles, dysentery, and scorpion poison.[17] It has antipyretic and antirheumatic properties and used as an anthelmintic for ascariasis, to treat scabies and control dental caries.[18] In the present study, three medicinally important plants were selected and analyzed for its antimicrobial activity and phytochemical constituents.

MATERIALS AND METHODS

Collection and Preparation Leaf Samples

The leaves of the medicinal plants, namely *Couroupita guianensis*, *Morinda tinctoria,* and *Tabernaemontana divaricata,* were collected in and around Coimbatore. Fresh leaves were washed in tap water and dried separately under shade to get dry. Then, it was powdered coarsely and stored in room temperature until use.[19]

Extract Preparation

The leaf powder of three plants was taken separately in three different conical flasks and soaked in petroleum ether to remove fats and chlorophylls for 24–48 h and it was filtered. The filtered materials were packed in Soxhlet apparatus and the solvent methanol was allowed to run in the apparatus. After extraction was completed, it was allowed to dry to get the crude extract. This crude extract of the leaves was stored in refrigerator until use.

Preparation of the Culture

Bacterial culture

Five different clinically isolated bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae,* and *Proteus vulgaris,* were collected from KMCH Hospital, Coimbatore, and it was subcultured in nutrient broth at 37°C for 24 h before use. Each bacterial growth was compared with 0.5 McFarland standard solution (containing about 1.5 × 10^8 CFU/mL) before inoculation.[19]

Fungal culture

Five different clinically isolated fungi, namely *Trichophyton rubrum*, *Penicillium chrysogenum*, *Aspergillus niger*, *Candida albicans,* and *Cryptococcus neoformans,* were collected from KMCH Hospital, Coimbatore, and it was subcultured in Sabouraud dextrose broth for 5–7 days in room temperature. Then, it was filtered to remove the mat and the filtrate was stored aseptically in refrigerator until use.[20]

Antibacterial Activity

Antibacterial activity was performed by well diffusion method. Mueller-Hinton agar plates were prepared aseptically and the surface of the agar was swabbed with bacterial culture and allowed to dry for few minutes.[21] Six wells (6 mm in size) were prepared using sterile cork borer. The center well was loaded with 100 µL ampicillin (positive control) and the rest four wells were loaded with different concentration of leaf extracts ranges from 25 µL to 100 µL and the fifth well was loaded with dimethyl sulfoxide (DMSO) and was served as a negative control. The plates were incubated at 37°C for 24 h.

Mycelial Growth Inhibition

Seeded agar technique was performed to determine mycelial growth inhibition of fungi.[22] Different concentrations of the leaf extracts ranges from 250 µg/mL to 1250 µg/mL were mixed with Sabouraud dextrose agar medium and plated aseptically. After it gets solidified, standardized 1 µL of fungal spores (containing 1 × 10^7 spores/mL) were inoculated at the center of the plate. Fluconazole (1 mg) plate served as a positive control and 2% DMSO plate served as a negative control. Then, these plates were incubated at room temperature for 5–7 days.[23]

Mycelial growth inhibition percentage was calculated by applying the formula,[24]

$$\text{Mycelial growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

The IC_{50} (half maximal inhibitory concentration) was attained using GraphPad Prism software.

Phytochemical Analysis

Standard procedureswere performed for the qualitative analysis of phytochemicals.[25-27]

RESULTS

Antibacterial Activity

The antibacterial activity of *C. guianensis*, *M. tinctoria,* and *T. divaricata* leaf extracts was summarized in Table 1. Among all tested bacteria, *S. aureus* only extends equal growth inhibition (16 mm) against both positive controls (ampicillin) and methanolic leaf extract of *C. guianensis* (highest concentration of extract) 100 µg/mL. In negative control (DMSO), no growth inhibition was found both in bacterial and fungal culture.

Mycelial Growth Inhibition

Percentage of the mycelial growth inhibition of three leaf extracts was calculated and the graph was plotted to determine the IC_{50} value. All tested fungi have different IC_{50} value against tested leaf extracts and the IC_{50} value of *C. neoformans* was not detected. The inhibition rate of mycelial growth was expressed in regression equation, represented in Table 2. *C. guianensis* possess high mycelial inhibition against *T. rubrum* followed by *A. niger* with 0.95 and 0.92 R^2 value, respectively. Similarly, *M. tinctoria* exhibits 0.92 R^2 value against *P. chrysogenum*.

Mycelial growth inhibition of three different leaf extracts was represented in Figure 1a-c.

Phytochemical Analysis

Qualitative analysis of phytoconstituents of three different leaf extracts was represented in Table 3.

DISCUSSION

In antibacterial activity, *C. guianensis* leaf extract possesses maximum growth inhibition against *S. aureus* (16 mm) in highest concentration (100 µg/mL) of methanolic leaf extract followed by *E. coli* (15 mm) and *S. pyogenes* (12 mm). *M. tinctoria* exhibits maximum growth inhibition against *K. pneumoniae* (5 mm) in highest concentration (100 µg/mL) of extract followed by *E. coli*, *S. pyogenes,* and *P. vulgaris* (2 mm). *T. divaricata* exhibits maximum growth inhibition against all tested bacteria (3 mm) in highest concentration of extract (100 µg/mL) except *P. vulgaris* (2 mm).

In the previous study Regina and Rajan,[28] reported that the maximum activity was recorded from methanolic leaf extract of *C. guianensis* (200 mg) against *E. coli* (29 mm)[29] followed by *S. aureus* (12 mm). Sivakumar *et al*.[30] reported that acetone and dichloromethane leaf extract of *C. guianensis* (100 mg) exhibits maximum inhibitory level against *S. aureus* (19 mm and 28 mm), respectively. Dixon *et al*.[16] Reported that chloroform leaf extract (100 mg) possesses maximum inhibition in *S. aureus* (16 mm) and no growth inhibition was found against *E. coli* and *K. pneumoniae* in methanol, ethanol, and chloroform leaf extracts.

In mycelial growth inhibition, *C. guianensis* (1000 µg/mL) possess complete growth inhibition against *T. rubrum* which shows similar activity in fluconazole (1000 µg/mL) followed by *P. chrysogenum* which shows complete growth inhibition (1250 µg/mL), and it was better than fluconazole. *M. tinctoria* methanolic

Table 1: Antibacterial activity of methanolic leaf extract of three different plants

Medicinal plants	Concentration of extracts in µg/µL	Zone of inhibition (mean±SEM)				
		Escherichia coli	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*	*Proteus vulgaris*
Couroupita guianensis	25	10±0.81	9±0.47	-	-	-
	50	12±0.81	12±00	-	-	-
	75	13±0.47	14±0.47	9±00	-	-
	100	15±0.47	16±0.81	12±0.81	-	-
	Amp	38±0.81	16±0.47	21±0.47	17±0.47	30±00
Morinda tinctoria	25	-	-	-	-	-
	50	-	-	-	-	-
	75	1±0.47	-	-	3±0.47	-
	100	2±0.47	-	2±0.81	5±0.81	2±0.47
	Amp	12±0.81	12±00	10±0.47	10±0.81	15±0.47
Tabernaemontana divaricata	25	-	1±00	-	-	-
	50	1±0.47	2±0.47	1±00	1±0.81	1±0.47
	75	2±00	2±0.47	2±0.47	2±0.81	1±0.81
	100	3±0.47	3±0.81	3±0.81	3±0.81	2±0.47
	Amp	12±0.47	11±0.81	10±0.81	10±0.47	16±0.47

-: No inhibition, Amp: Ampicillin, SEM: Standard error mean

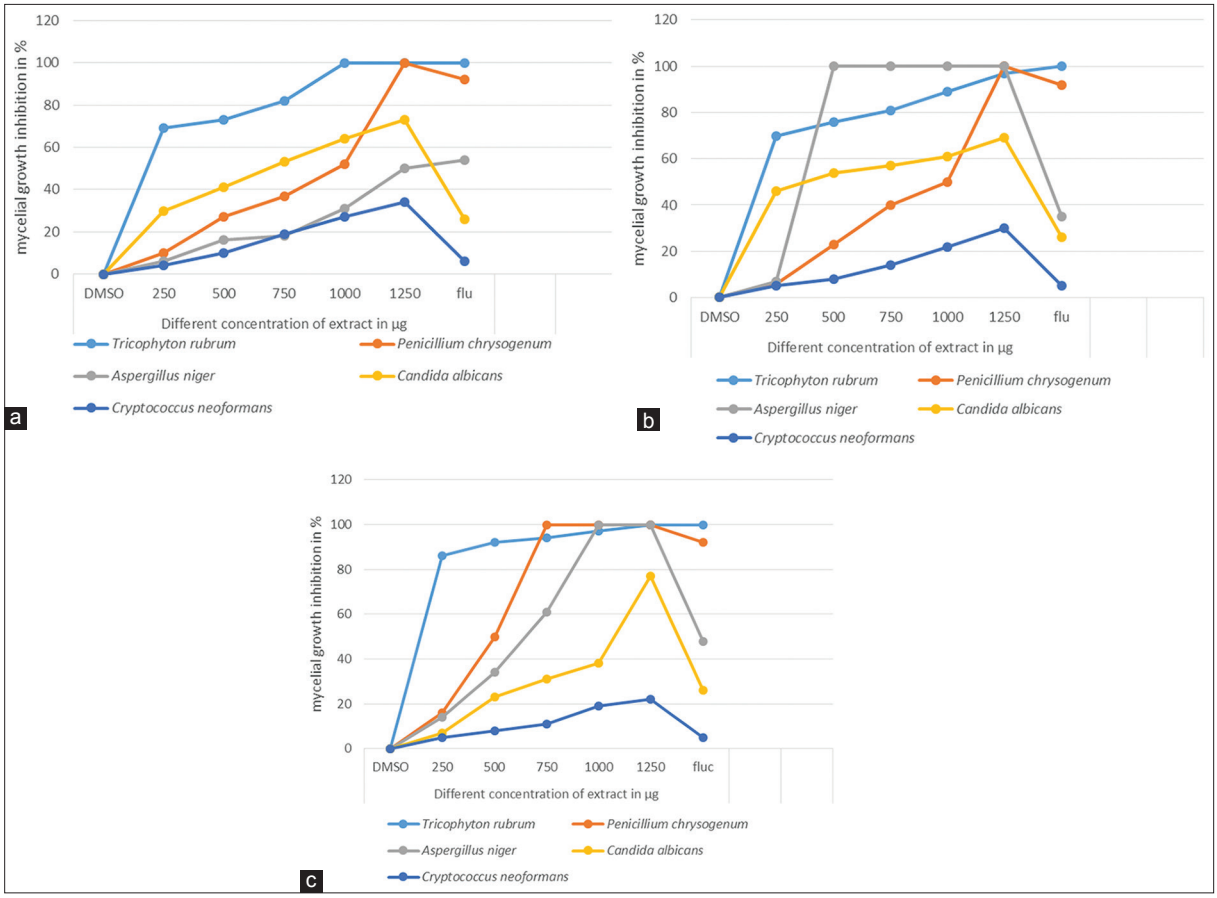

Figure 1: (a) Mycelial growth inhibition of methanolic leaf extract of *Couroupita guianensis*. (b) Mycelial growth inhibition of methanolic leaf extract of *Morinda tinctoria*. (c) Mycelial growth inhibition of methanolic leaf extract of *Tabernaemontana divaricata*

Table 2: Inhibition of mycelial growth against three different leaf extracts

Medicinal plants	*Trichophyton rubrum*	*Aspergillus niger*	*Penicillium chrysogenum*	*Candida albicans*	*Cryptococcus neoformans*
Couroupita guianensis					
R^2 value	0.95	0.92	0.71	0.33	0.33
IC_{50}	180	1250	970	700	ND
Morinda tinctoria					
R^2 value	0.69	0.22	0.92	0.17	0.32
IC_{50}	180	370	1000	380	ND
Tabernaemontana divaricata					
R^2 value	0.50	0.56	0.77	0.51	0.33
IC_{50}	140	660	290	1080	ND

R^2: Coefficient of determination, IC_{50}: Half the minimum inhibitory concentration, ND: Not detected

leaf extract (1250 µg/mL) possesses complete mycelial growth inhibition against *A. niger* and *P. chrysogenum. T. divaricata* methanolic leaf extract (1250 µg/mL) exhibits complete mycelial growth inhibition against *T. rubrum*, *A. niger,* and *P. chrysogenum* which was better than fluconazole inhibition.

In the present study, *C. guianensis* exhibits high mycelial growth inhibition against *T. rubrum*, but Devi *et al*.[31] reported that *C. guianensis* does not show any inhibitory activity against *T. rubrum.* Followed by *T. rubrum*, the growth inhibition was better with *A. niger*. Kamalakannan *et al*.[29] Reported that methanolic extract of *C. guianensis* (200 mg) exhibited high antifungal activity in *A. niger* (19 mm). Regina and Rajan[28] Reported that chloroform extract of *C. guianensis* showed significant antifungal activity against *C. albicans* (8 mm). Singh *et al*.[19] Reported that methanol, ethanol, and chloroform leaf extract (100 mg/mL) possesses no growth inhibition against *C. albicans*. Lavanya and John[32] stated that three different extracts (petroleum ether, chloroform, and ethanol) of *C. guianensis* leaf (10 mg) were more effective against *T. rubrum* (19 mm),

Table 3: Phytochemical analysis of methanolic leaf extract

Name of the phytoconstituents	*Couroupita guianensis*	*Morinda tinctoria*	*Tabernaemontana divaricata*
Alkaloids			
Mayer's test	-	+	-
Wagner's test	-	+	+
Flavonoids			
Alkaline test	-	-	+
Lead acetate test	-	-	-
Tannin			
Gelatin test	+	+	+
Terpenoids			
Salkowski's test	+	-	-
Glycosides	-	-	+
Phenolic compounds	+	-	-
Saponin	-	-	+

+: Presence, -: Absence

C. albicans (16 mm), and also possess lesser effect on *C. neoformans* (13, 11, and 15 mm), respectively.

Qualitative analysis of phytochemicals revealed the presence of tannin, terpenoids, and phenolic compounds in *C. guianensis* leaf extract, alkaloids and tannin in *M. tinctoria*, and alkaloid, flavonoids, tannin, glycosides, and saponin in *T. divaricata* leaf extract. In the previous study, Alagesaboopathi[17] reported thatmethanolic extract of *C. guianensis* showed the presence of saponin and the absence of alkaloids.

CONCLUSION

All three leaf extracts exhibit complete mycelial growth inhibition against most of the tested fungi which were better than fluconazole. *T. divaricata* possess better mycelial growth inhibition, but the gradual increase of fungal mycelium inhibition was achieved by *C. guianensis*. Antibacterial activity was efficient in *C. guianensis* when compared with other two plant extracts. Hence, from the result, it was concluded that the antimicrobial activity was highly significant in *C. guianensis* methanolic leaf extract. Moreover, this activity may be due to the presence of phytochemicals such as phenolic compounds, tannin, and terpenoids. Further study was required to identify the compound responsible for the activity and *in vivo* study can be carried out to check the toxicity.

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