Journal of Current Medical Research and Opinion
Received 14-10-2024 | Revised 15-10-2024 | Accepted 09-11-2024 | Published Online 10-11-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-11-3 ISSN (O) 2589-8779 | (P) 2589-8760
CMRO 07 (11), 3714-3723 (2024) www.cmro.in

Research Article

Antifungal Activity and Phytochemical Analysis of Leaves Extract of Medicinal Plant *Cassia tora* Using GC/MS

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Abstract:

The extensive evidence for the many pharmacological and therapeutic possibilities may be provided by the several activities of Cassia tora. This study used gas chromatography–mass spectrometry to examine the antifungal activity and phytochemical profile of an extract from the medicinal Cassia tora plant. The resulting crude extracts were further refined with Whatman No. 1 filter paper and kept at 4 °C for future reference. A rotary evaporator operating under vacuum at 40 °C achieved the desired concentration. To determine the chemicals' identities, we used industry-standard techniques to compare their mass spectra to those in the NIST/Wiley internal reference mass spectra library. The antifungal activity of an extract from Cassia tora leaves was tested using the cup plate method, which involves 90% methanol. Dry heat in a hot air oven was used to sterilise petri plates for 1.5 hours at 160 °C. Laminitol, Methyl 1-fluoro-4-oxocyclohexane-1-carboxylate, Hexadecanoic acid, alpha-Linolenic acid, 3,7,11,15-Tetramethylhexadec-1-en-3-ol, alpha-Tocopherol acetate, 6-Dodecanone, 5,8-diethyl-7-hydroxy, Methyl beta-D-glucopyranoside, 1,3-Dioleoyl-2-palmitoylglycerol, 2-propylpentyl 14-methylpentadecanoate, 8-Ethylquinoline-3-carboxylic acid, 1-Allyl-4-methoxy-2,3-dimethylbenzene. Methanol, Ethyl acetate, and Ethanol were Candida albicans, (15.00 ± 0.31, 18.55 ± 0.39 and 21.00 ± 0.43 respectively), Candida glabrata (23.00 ± 0.49, 19.08 ± 0.35 and 26.05 ± 0.51 respectively), Trichophyton rubrum (13.00 ± 0.31, 20.74 ± 0.41 and 24.77 ± 0.50 respectively), Microsporum canis (23.09 ± 0.44, 15.95 ± 0.31 and 17.22 ± 0.36 respectively), Aspergillus niger (10.00 ± 0.27, 09.94 ± 0.18 and 18.45 ± 0.37 respectively), Alternaria Alternaria (11.57 ± 0.21, 08.96 ± 0.17 and 15.90 ± 0.36 respectively), and standard antifungal [Voriconazole (VCZ) and Fluconazole (FCZ)] were (29.00 ± 0.54 and 31.79 ± 0.59) respectively.

Keywords: Antifungal Activity, Medicinal Plant, GC/MS, *Cassia tora*

Introduction:

Cassia tora is a small shrub that grows weed-like in almost all Asian countries. A large and economically important plant family, the leguminosae is sometimes known as the bean, pea, or legume family. The seeds of legume plants eventually mature into pods [1-3]. Because they are rich in protein, fibre, carbohydrates, and minerals, legumes should be a regular part of any healthy diet. Overall, legumes are ideal for animals because of their nutritional profile, which allows them to meet dietary standards. A food with medicinal and health-promoting qualities, legumes have long been recognised. The subfamily Casual pinioideae consists of the lentil Cassia tora. An annual herb found growing wild in the wasteland of India, especially during the rainy season, that is 30-90cm tall orginates its name from the sinhalese language and is called Tora. Its weed like wild crop Cassia tora is found in most parts of India. Cassia tora has the usage of all its components like leaves, roots and seeds that make them function to serve their specific purpose. Some of these compounds which are in the active state in cassia tora include anthraquinone, quacetin, chrysophenol, emodin and rhein. Cassia tora has a strong effect on antimutagenic. This plant has sensation characteristics by containing anthraquinone acting as a fluorescence sensor or fluorophores [4, 5]. It contains one of the maximum effectuating antifungal formulation, “Dadhughnavati”, an Ayurveda medical treatment. Because most of the pharmaceutical market depends on medicinal plants in developing pharmaceutical chemicals, those who work in the biotechnology field are very much concerned in this medicinal plants. A majority of plural-constituents employed in biochemicals in drugs, fragrances, food dyes/pigmentation, and flavours come from plant sources [6, 7]. Whole crude plant extracts, containing a cocktail of various phytochemical constituents (plant secondary metabolites) were often used in the formulations of most of the claimed herbal medications and their derivatives. There is a large range of variation in the chemical properties of these components among species [8]. The gas chromatography–mass spectrometry (GC–MS) technique is a promising tool for determining the concentration of active ingredients in medicinal, cosmetic, pharmaceutical, and food-related herbs. This study used gas chromatography–mass spectrometry to examine the antifungal activity and phytochemical profile of an extract from the medicinal Cassia tora plant.

Materials and Methods:

Plant sample collection:

The medical herbs office in Hillah city, Iraq, provided the laboratory with the leaves of Casia tora, which were harvested in good condition. After a good washing with running water, the leaves were let aside to dry naturally at room temperature. Prior to conducting more research, the dried plant samples were ground into a powder and sealed in plastic bags.

Methods for making a plant extract:

A series of ethanol extractions were performed on the dried powder. Ten grammes of the dehydrated and powdered plant material were extracted for six to eight hours at a temperature lower than the solvents' boiling points using a Soxhlet equipment and one hundred sixty millilitres of each ethanol. After collecting the crude extracts, they were filtered using Whatman No. 1 paper and kept at 4 °C till later. A rotary evaporator operating under vacuum at 40 °C achieved the desired concentration.

Gas chromatography – mass spectrometry analysis (GC-MS):

Chromatography using Gas A Perkin Elmer, Claurs 680 GC paired with a PE SQ-8 C mass analyser was used to conduct the mass spectrometry analysis. A capillary column with a particle size of 0.25 µm and dimensions of 30 M X 0.25 mm was employed. The temperature programming for the carrier gas, helium, begins at 400C, holds for 5 minutes, then ramps up to 2600C at a rate of 120C/min. After that, it is held isothermally for 5 minutes. Using an autosampler, the injector was set to 2500C with a carrier flow rate of 1 mL/min. The injector was a Programmable Split-Splitless Injector (PSSI) in

split mode with a volume of 1 µl. In electron ionisation mode, with an ionisation voltage of 70eV, the sample components were ionised with an ion source temperature of 1800C and a transfer line temperature of 2000C [9, 10]. From 50 to 550 amu, the mass range was utilised. In accordance with established protocols, the chemicals were identified by comparing their mass spectra to those in the NIST/Wiley internal reference mass spectra database.

Assessing the efficacy of antifungal agents:

The antifungal activity of an extract from Cassia tora leaves was tested using the cup plate method, which involves 90% methanol. Dry heat in a hot air oven was used to sterilise petri plates for 1.5 hours at 160 °C. The distilled water was used to create Sabouraud's dextrose agar. After inserting a non-adsorbent cotton plug, 20 millilitres of molten agar was added to the test tube. We autoclaved all of the test tubes to ensure their sterility. After 30 minutes, the plates were allowed to cool to room temperature. For every agar plate, a No. 4 cork borer was used to make a hole. Separate agar plates were treated with 0.1 ml of plant extract from each plant, and the resulting diluted solution was 30 mg/ml in 90% methanol. For the hour prior to incubation, the plates were placed in the fridge to allow for diffusion. The samples of Candida albicans, Candida glabrata, Trichophyton rubrum, Microsporum canis, Aspergillus niger, and Alternaria alternaria were left to come to room temperature for one hour. A three-day incubation period was subsequently elapsed in an incubator set at 30-32 ºC. The petri dishes were examined and the diameters of the inhibition zones were noted at the conclusion of the three-day period. A 90% solution of methanol served as the control group's negative counterpart. The conventional antibiotics employed were voriconazole (VCZ) and fluconazole (FCZ) (the positive control). The average size of the inhibition zones was determined by repeating the trials three times.

Statistical Analysis:

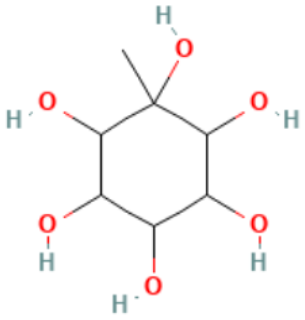
We utilised SPSS 19.0 (IBM, New York, NY, USA) for statistical analysis and Tukey's honestly significant differences (HSD) test to compare the average mean values with a confidence interval of 95% or 99%. The ANOVA analysis of variance was carried out. Statistical significance was determined by a p-value lower than 0.05.

Results and Discussion:

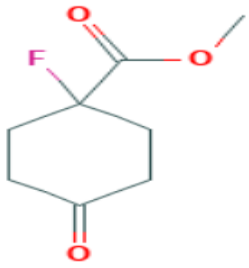
There is great therapeutic value in the little shrub Cassia tora Linn. Traditional communities in the Amarkantak area of the Anuppur district of Madhya Pradesh have long relied on the plant's young leaves and pods for a delicious vegetable curry. For medicinal purposes, including treating malaria and reducing neurotoxicity, the local indigenous healers rely on this herb. Aiming to establish a connection between the plant's purported pharmacological activity and its primary phytochemicals, the current investigation set out to do just that. The primary components of the Cassia tora methanol extract were determined using the GCMS-NIST database [11, 12]. A search was conducted on Pubchem to match the details of important phytochemicals with the available database. We looked through the literature for information on the pharmacological activity of the main ingredients. We used the GCMS-NIST library to identify twelve main chemicals from the methanolic extract of Cassia tora leaves. It has been observed that laminitol, the most abundant phytoconstituent, has antifungal activity. It is reasonable to employ Cassia tora for ethnopharmacological purposes in this area since it contains components with antifungal action. What were these chemicals: Laminitol, Methyl 1-fluoro-4-oxocyclohexane-1-carboxylate, Hexadecanoic acid, alpha-Linolenic acid, 3,7,11,15-Tetramethylhexadec-1-en-3-ol, alpha-Tocopherol acetate, 6-Dodecanone, 5,8-diethyl-7-hydroxy, Methyl beta-D-glucopyranoside, 1,3-Dioleoyl-2-palmitoylglycerol, 2-propylpentyl 14-methylpentadecanoate, 8-Ethylquinoline-3-carboxylic acid, 1-Allyl-4-methoxy-2,3-dimethylbenzene (Table 1).

Table 1. Bioactive Chemical secondary metabolites of ethanolic extract of *Cassia tora*.

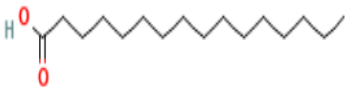
Compounds	Molecular Formula	Molecular Weight
Laminitol	$C7H_{14}O_6$	194.18 g/mol
Methyl 1-fluoro-4-oxocyclohexane-1-carboxylate	$C8H_{11}FO_3$	174.17 g/mol
Hexadecanoic acid	$C16H_{32}O_2$	256.42 g/mol
alpha-Linolenic acid	$C18H_{30}O_2$	278.4 g/mol
3,7,11,15-Tetramethylhexadec-1-en-3-ol	$C20H_{40}O$	296.5 g/mol
alpha-Tocopherol acetate	$C_{31}H_{52}O_3$	472.7 g/mol
6-Dodecanone, 5,8-diethyl-7-hydroxy	$C_{16}H_{32}O_2$	256.42 g/mol
Methyl beta-D-glucopyranoside	$C_7H_{14}O_6$	194.18 g/mol
1,3-Dioleoyl-2-palmitoylglycerol	$C_{55}H_{102}O_6$	859.4 g/mol
2-propylpentyl 14-methylpentadecanoate	$C_{24}H_48O_2$	368.6 g/mol
8-Ethylquinoline-3-carboxylic acid	$C_{12}H_{11}NO_2$	201.22 g/mol
1-Allyl-4-methoxy-2,3-dimethylbenzene	$C_{12}H_{16}O$	176.25 g/mol



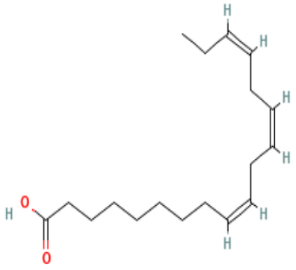
Laminitol



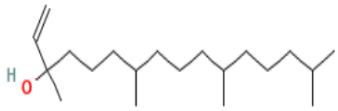
Methyl 1-fluoro-4-oxocyclohexane-1-carboxylate



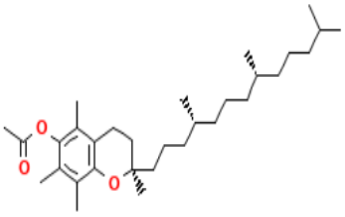
Hexadecanoic acid



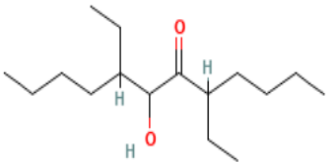
alpha-Linolenic acid



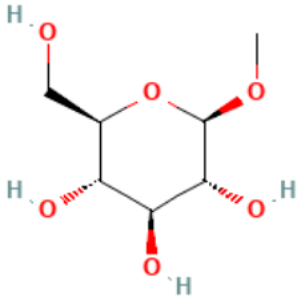
3,7,11,15-Tetramethylhexadec-1-en-3-ol



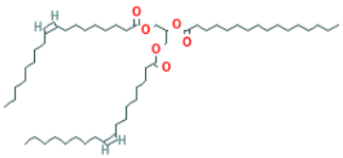
alpha-Tocopherol acetate



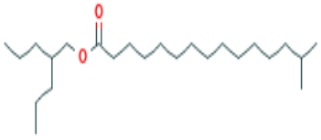
6-Dodecanone, 5,8-diethyl-7-hydroxy



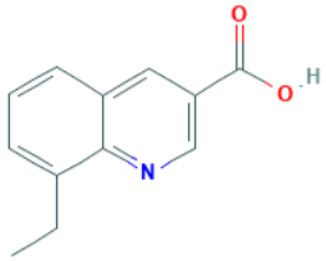
Methyl beta-D-glucopyranoside



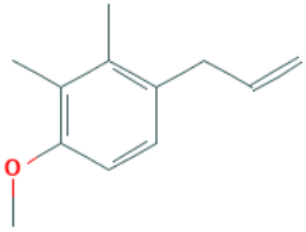
1,3-Dioleoyl-2-palmitoylglycerol



2-propylpentyl 14-methylpentadecanoate



8-Ethylquinoline-3-carboxylic acid



1-Allyl-4-methoxy-2,3-dimethylbenzene

Comparing the antifungal efficacy of classic antifungals [Voriconazole (VCZ) and Fluconazole (FCZ)] with that of secondary metabolites isolated from Cassia tora leaves (Figure 1-5): Methanol, Ethyl acetate, and Ethanol were Candida albicans, (15.00 ± 0.31, 18.55 ± 0.39 and 21.00 ± 0.43 respectively), Candida glabrata (23.00 ± 0.49, 19.08 ± 0.35 and 26.05 ± 0.51 respectively), Trichophyton rubrum (13.00 ± 0.31, 20.74 ± 0.41 and 24.77 ± 0.50 respectively), Microsporum canis (23.09 ± 0.44, 15.95 ± 0.31 and 17.22 ± 0.36 respectively), Aspergillus niger (10.00 ± 0.27, 09.94 ± 0.18 and 18.45 ± 0.37 respectively), Alternaria Alternaria (11.57 ± 0.21, 08.96 ± 0.17 and 15.90 ± 0.36 respectively), and standard antifungal [Voriconazole (VCZ) and Fluconazole (FCZ)] were (29.00 ± 0.54 and 31.79 ± 0.59) respectively.

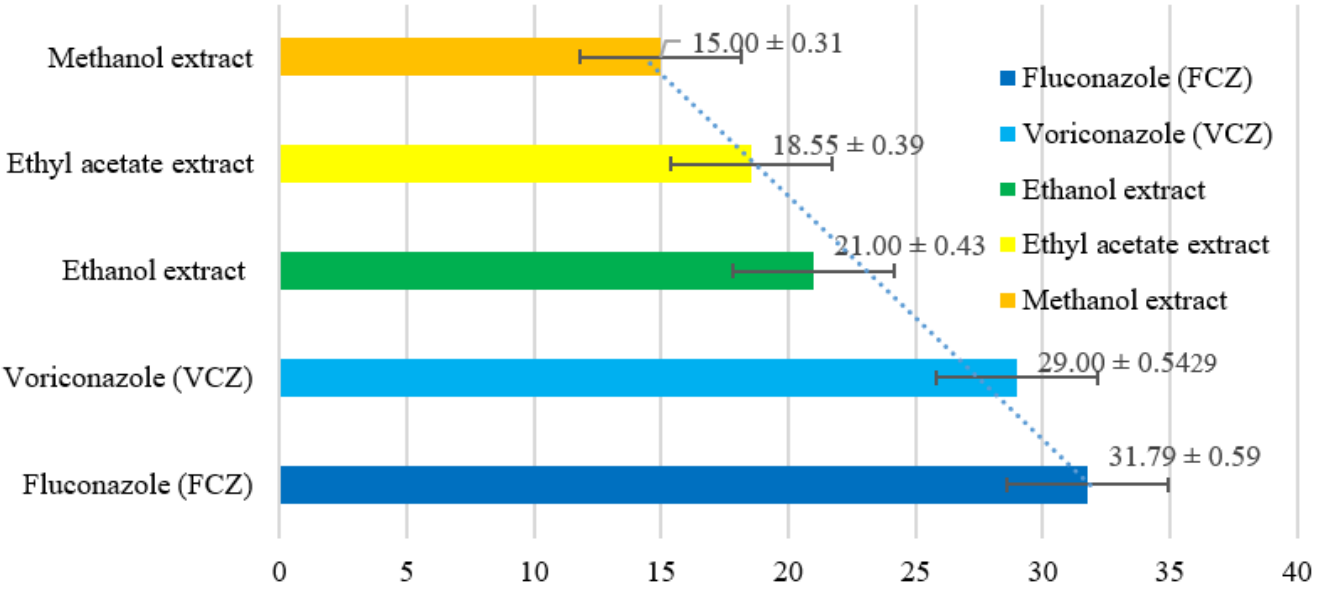


Figure 1. Antifungal activity of secondary metabolites of leaves extract of *Cassia tora* and standard antifungal activity [Voriconazole (VCZ) and Fluconazole (FCZ)] against *Candida albicans*

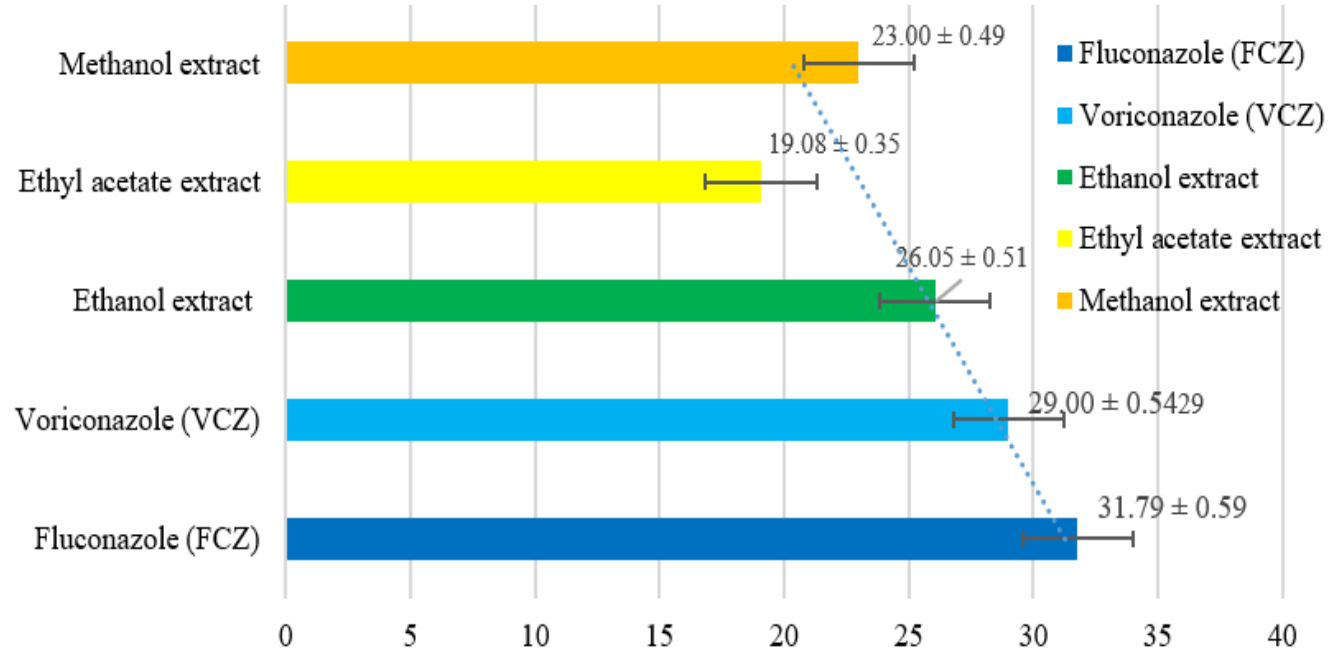


Figure 2. Antifungal activity of secondary metabolites of leaves extract of *Cassia tora* and standard antifungal activity [Voriconazole (VCZ) and Fluconazole (FCZ)] against *Candida glabrata*

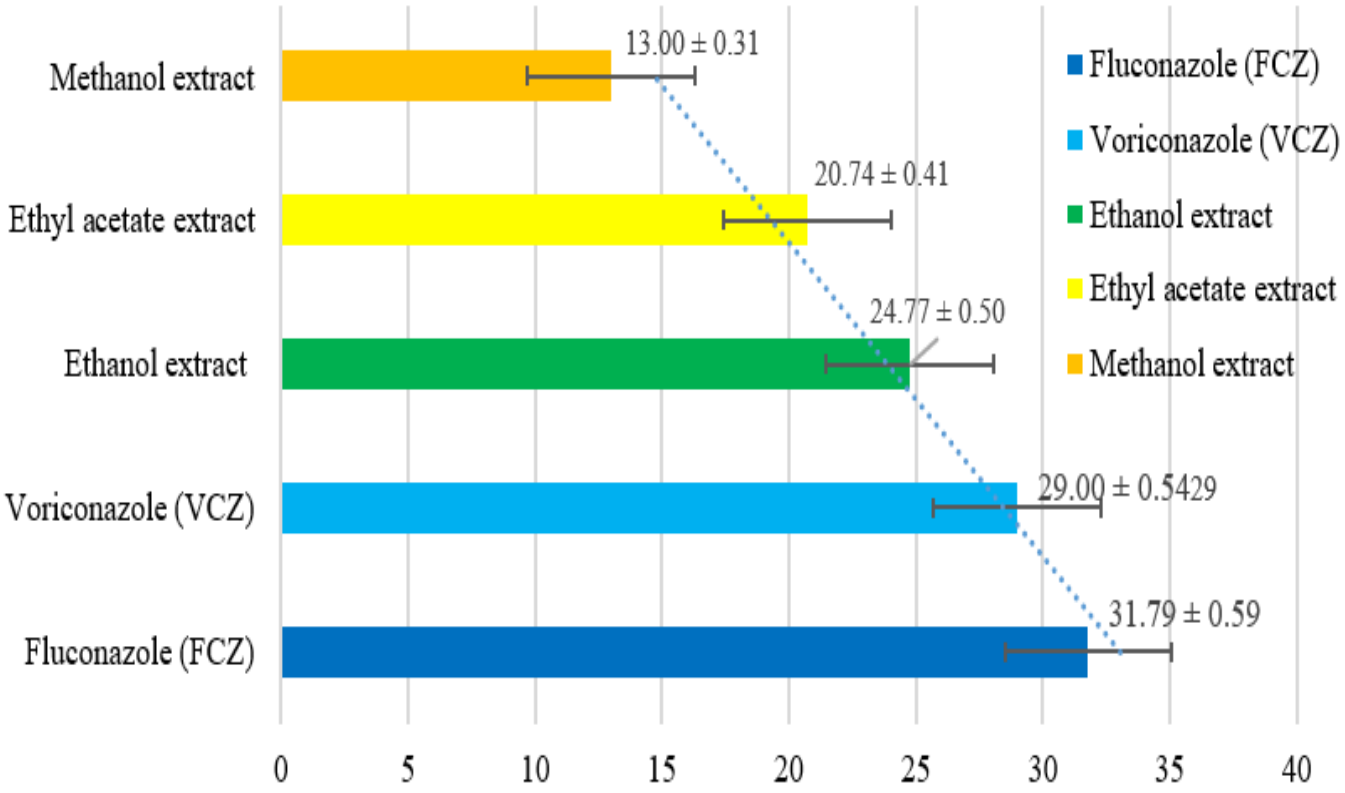


Figure 3. Antifungal activity of secondary metabolites of leaves extract of *Cassia tora* and standard antifungal activity [Voriconazole (VCZ) and Fluconazole (FCZ)] against *Trichophyton rubrum*

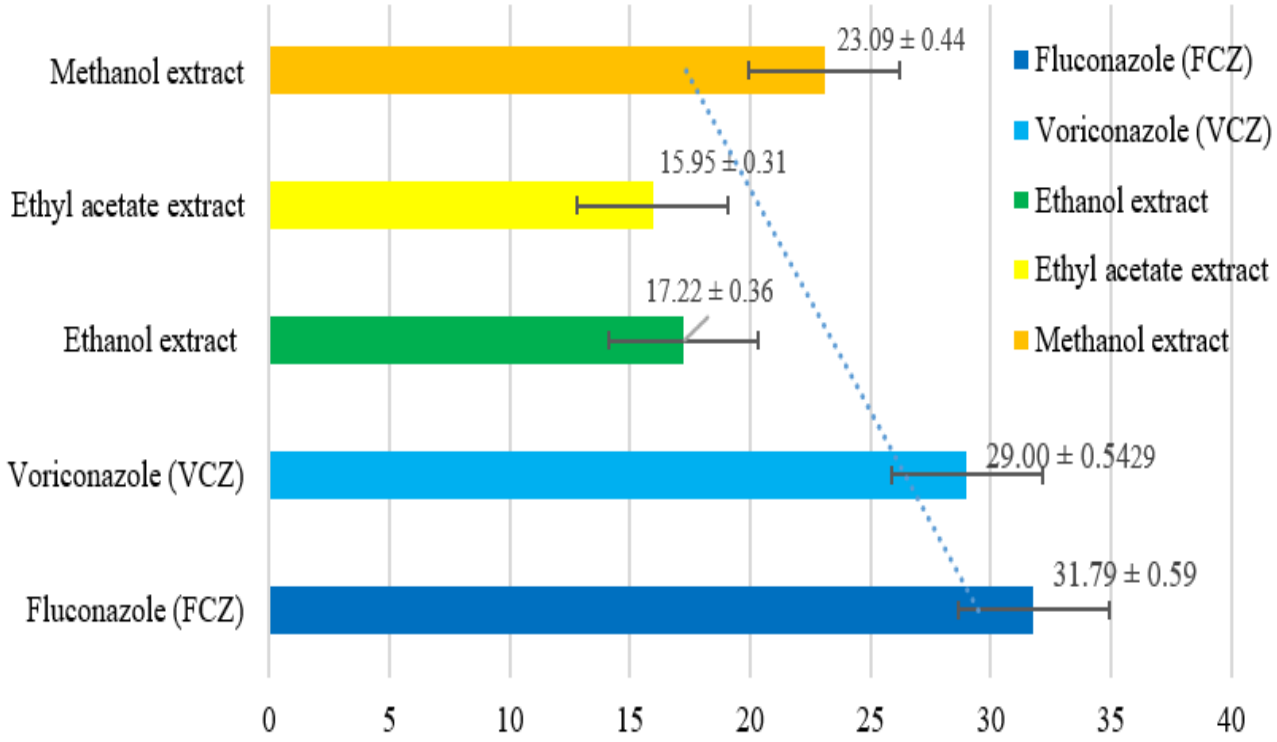


Figure 4. Antifungal activity of secondary metabolites of leaves extract of *Cassia tora* and standard antifungal activity [Voriconazole (VCZ) and Fluconazole (FCZ)] against *Microsporum canis*

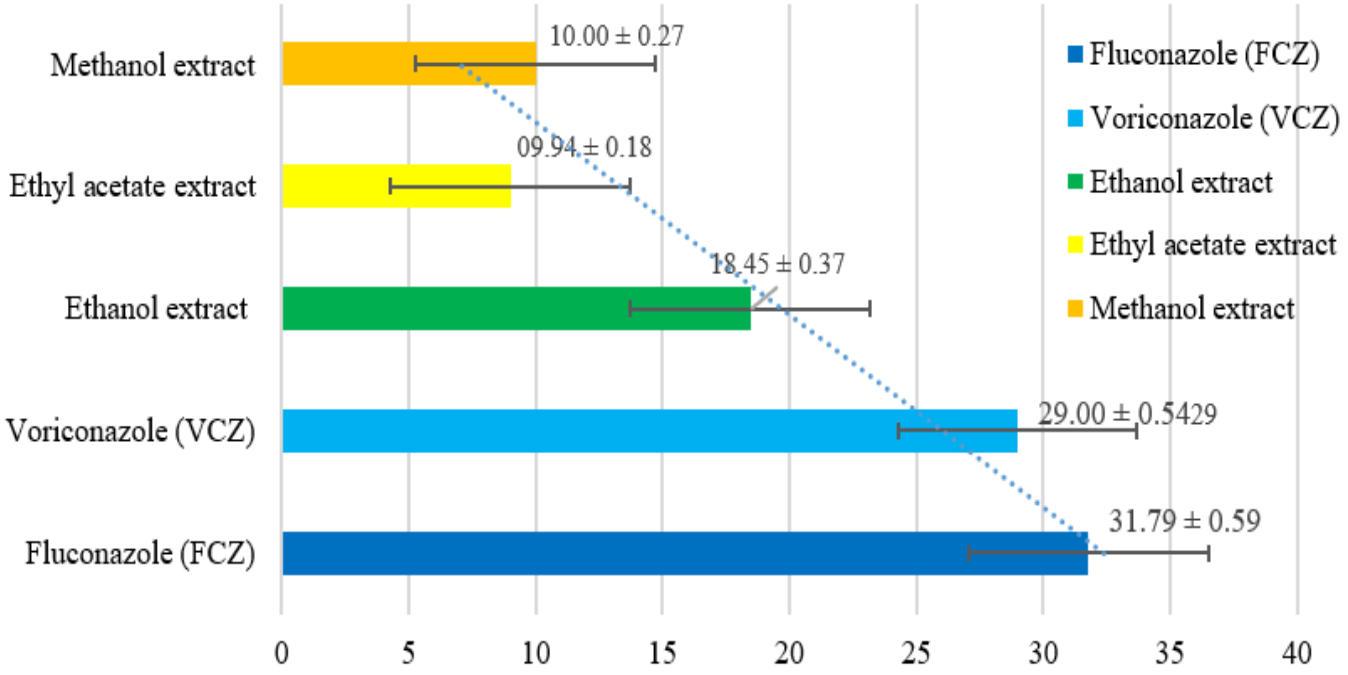


Figure 5. Antifungal activity of secondary metabolites of leaves extract of *Cassia tora* and standard antifungal activity [Voriconazole (VCZ) and Fluconazole (FCZ)] against *Aspergillus niger*

Contrary to expectations, even with new antifungal drugs being synthesised on the market and development in medicine, fungal diseases have remained among the most life-threatening and disabling. Our health care providers are headed for a disaster on how to handle the increased rates of occurrence of fungal infections. Like Candida albicans, opportunistic pathogens of AIDS are prevalent and lead to an emergent condition thus causing candidiasis with global reach. In fact, the last twenty years have witnessed an apparent epidemic of different forms of fungal diseases which can be attributed to increased presence of immunocompromised individuals. Those fatalities are especially vulnerability significant for opportunistic systemic mycoses. Infections of the skin and nails can be caused by Candida albicans, a fungus that lives in the mouth and intestines and can spread from there. Babies with oral thrush have produced cutaneous moniliasis (candidiasis) over the nipple in nursing mothers and balanoposthitis in hundreds of women with monilial vaginitis [13–15]. Although now there are several effective medicines which help treat fungal diseases, these medicines have several inconveniences: side effects, the possibility of resistance and insufficient sensitivity to certain infections. Subsequently, discovering effective medications for such infections is of paramount significance since multiple drug resistant Candida albicans strains are being reported in several parts of the world. Therefore, there is concern with new information in the traditional system of medicine with intention of developing improved drugs against microbial infections. In many rural regions of a number of developing countries it is almost an open secret that some ailments can be treated using plant derived products [16-19]. In most cases, traditional healers are cheaper or, at least, occasionally offer a remedy that is superior to a modern pharmacological preparation. The three Cassia leaf extracts tested here were found to suppress the growth of five different types of fungus. These results back up the traditional use of these plants as a remedy for skin disorders and ringworm. Opportunistic P. marneffei infections are widespread among HIV-positive people in northern Thailand and define AIDS. Penicilliosis marneffei can be effectively treated with these three Cassia species. There have been numerous reports of C. alata's antibacterial activity against human infections. A 5% water-based extract of C. alata leaves containing rhein, emodol, 4,5-

dihydroxy-1-hydroxymethylanthrone, and 4,5-dihydroxy-2-hydroxymethylanthraquinone inhibited the growth of several yeasts and dermatophytes. We investigated the antifungal activity of C. alata leaf extract using petroleum ether, hot 85% ethanol under reflux, and dermatophytes, as well as Candida albicans, Aspergillus fumigatus, Aspergillus flavus, Mucor spp., Rhizopus spp., and Trichophyton mentagrophytes, T. rubrum, and M. gypseum. The contaminating fungi were not significantly affected by the 20% w/v crude extract, however the growth of dermatophytes was entirely prevented at 2.5 and 3% crude extract, respectively. A majority of the studied extracts showed anti-dermatophytic activity, regardless of whether they were extracted using water, alcohol, hexane, petroleum ether, or ethyl acetate [20, 21]. Antibacterial studies for C. fistula are few and far between. Among the antifungal components of C. tora, the most potent against dermatophytes was chrysophanic acid-9-anthrone, which was isolated from the defatted seed. The results show that all three of these Cassia leaf extracts are antifungal. Of the three Cassia species, C. fistula was most effective against P. marneffei, while C. alata was most effective against T. rubrum and M. gypseum. Also investigated were the effects of the extracts on the macroconidia of Mycoplasma gypseum. With the exception of C. alata and miconazole, the IC50 values calculated to inhibit conidial germination were higher than those calculated to prevent hyphal growth. Microscopical examination of the extracts' effects on macroconidia and fungal hyphae revealed, nevertheless, comparable morphological change, characterised by a shrunken and collapsed shape. They elaborated that this phenomena could be caused by cytoplasm loss as a result of cell wall leaks or changes to membrane permeability. Dermatophyte antifungal activity was also demonstrated by crude anthraquinones, rhein, and aloeemodine. The antibacterial activity of flavone glycosides from C. fistula seeds and C. tora chrysophanic-9 anthrone were observed in separate experiments. There may be a connection between cell fluid leaks and the antifungal properties of the Cassia species' leaf extracts. It is critical to identify the active ingredients in each extract and validate their mechanism for subsequent studies [23, 24]. Several researches stated that the death rate attributed to these fungi has also risen over the previous decades because of emergence of drug-resistance incidence and cross resistance among the isolated species. This is despite the increasing availability of antifungal drugs. Candida and Aspergillus species are the most common resistant fungi that have caused changes in susceptibility to the regular drugs and constant fungal infection. Despite that diversification extracts and some essential oils of C. colocynthis in several studies have been proved to exhibit an impact on the growth of some medically important fungus [25]. Phenolic molecule such as tannin and flavonoids alter the make up and functionality of the cell and cause cell membrane protein binding and precipitation. This leads to the release of the components of the cell, and this in a way results to cell death [26, 27]. It would be possible these pathways it may have other way with reducing enzyme activity including protein binding or inhibition of formation of DNA/RNA. According to the identification of antifungal activities, one extract from Cassia tora was identified to have some potential. It can also be formulated into topical use for ailments which affect the skin such as eczema, dermatitis, itching and rashes. Further in vivo study is needed to confirm the findings of the current research.

Conclusion:

The current study tested the antifungal activity of a crude methanolic extract of Cassia tora leaves against many fungus species. This could explain why the MIC value was so high, at 2 mg/ml. Antifungal action at lower concentrations would likely be observed in a more refined formulation. It is necessary to conduct additional clinical and scientific trials on the specific fraction or isolate of the ethanolic Cassia tora leaf extract. The foundation of traditional medicine, medicinal plants, have been the focus of copious pharmacological research in recent decades. This

is due to the growing recognition of medicinal plants' potential as sources of novel therapeutic compounds and lead compounds for drug development. Consequently, twelve compounds were identified in Cassia tora using GC-MS analysis, proving the presence of bioactive chemicals. Laminitol, methyl 1-fluoro-4-oxocyclohexane-1-carboxylate, hexadecanoic acid, and alpha-Linolenic acid are some of the chemicals that have been found. This would also be useful for determining which Cassia tora leaf components are responsible for the antifungal effects.

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