UNIVERSITI PUTRA MALAYSIA

Cassia alata ANTIFUNGAL PROPERTIES: A SYSTEMATIC REVIEW

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In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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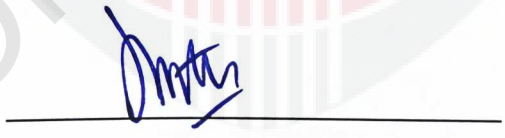
CERTIFICATION

It is hereby certified that I have read this project paper entitled "*Cassia alata* Antifungal Properties: A Systematic Review", by Nurul Afiqah Binti Lokman and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999-Final Year Project.



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DEDICATION

Allah SWT, The Almighty, The Most Merciful

My supportive supervisor,

Dr Wan Mastura Shaik Mohamed Mossadeq

My beloved parents,

Lokman Bin Abd Basit and Norazah Binti Ibrahim

My dearest siblings,

Nurul Ameera, Muhammad Amzar and Muhammad Afnan

Kindest friends,

Arinah Nabihah, Nurul Syamimie, Wan Hasyimah, Nur Hanisa and DVM2022.

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This thesis will not be completed without the strength given by Allah SWT. Praise to Him, this time was very different yet comforting for being able to stay close to my dearest family. It has been a journey of knowledge and a test of discipline to myself. Without Him, I would not be where I am today.

First and foremost, I would like to express my highest gratitude to my supervisor, Dr Wan Mastura Shaik Mossadeq for the guide and knowledge you have shared throughout until project's completion. To my dearest family, I thank everyone for enduring my hiatus, nothing compares to the warmth of comfort and support when I needed those the most.

To the girls whom I have been through thick and thin together, thank you for everyone's kind care and effort to lend a helping hand. To DVM2022, thank you for the immense support and guidance during this difficult time.

To those I lost during this pandemic and my dearest grandfather, this is for you.

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LIST OF ABBREVIATIONS

AD	Agar Dilution
AgNP	Silver Nanoparticle
AWD	Agar Well Diffusion
BD	Broth Dilution
DD	Disc Diffusion
GC-MS	Gas Chromatography-Mass Spectrophotometry
MFC	Minimum Fungicidal Activity
MIC	Minimum Inhibitory Concentration
ZOI	Zone of Inhibition

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kursus VPD 4999 - Projek Ilmiah Tahun Akhir.

SIFAT ANTIKULAT *CASSIA ALATA*: TINJAUAN SISTEMATIK

Oleh

Nurul Afiqah Binti Lokman

2021

Penyelia: Dr Wan Mastura Bt Shaik Mohamed Mossadeq

Cassia alata (semak kurap) digunakan secara meluas dalam perubatan tradisional kerana sifat antikulatnya. Pokok renek hiasan ini sering tumbuh subur dalam keadaan lembap di kawasan tropika. Aplikasi sifat antikulat *Cassia alata* (*C. alata*) dalam perubatan veterinar disemak secara sistematik. Semakan ini telah dijalankan mengikut protokol kajian Item Pelaporan Pilihan untuk Kajian Semula Sistematik dan Meta-analisis (PRISMA). Pencarian literatur dilakukan dengan mengenal pasti semua artikel jurnal yang berkaitan dengan topik daripada 3 pangkalan data iaitu Scopus, Science Direct dan CAB Direct. Sebanyak 313 artikel yang diterbitkan dari tahun 2000 hingga 2021 telah dipilih. Abstrak dan rujukan artikel ini kemudiannya dieksport ke EndNote X9 untuk penelitian selanjutnya. Berikutan proses saringan dan kelayakan, 30 kajian didapati layak, dan semua data yang berkaitan telah dijadualkan

menggunakan Microsoft Office Excel 2016. Semakan komprehensif artikel menunjukkan bahawa bahagian tumbuhan yang paling kerap digunakan ialah daun, diikuti dengan bunga, akar, kulit kayu, dan biji. Ekstrak organik (cth.: metanol dan etanol) didapati mempamerkan aktiviti antikulat yang lebih tinggi daripada pelarut lain. Kesan antikulat *C. alata* kebanyakannya telah diuji ke atas *Candida albicans* dan *Trichophyton mentagrophytes*. Aktiviti antikulat yang ditunjukkan oleh ekstrak *C. alata* adalah sama berkesan terhadap dermatofit dan yis. Walau bagaimanapun, terdapat variasi dalam keberkesanan aktiviti antikulat *C. alata* yang dilaporkan. Percanggahan dalam keberkesanan tumbuhan ini dalam kajian *in vitro* mungkin disebabkan oleh variasi dalam keadaan kajian dan/atau kepekatan ekstrak mentah yang digunakan. Oleh itu, penentuan sebatian fitokimia yang tepat yang bertanggungjawab terhadap kesan antikulat *C. alata in vitro* adalah wajar. Namun begitu, penggunaan nanopartikel logam bertutup *C. alata* sebagai agen antikulat untuk mengatasi rintangan antimikrob menjanjikan sesuatu yang baik pada masa ini.

Kata Kunci: *Cassia alata*; Antikulat; Veterinar; *In vitro*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

CASSIA ALATA ANTIFUNGAL PROPERTIES: A SYSTEMATIC REVIEW

By

Nurul Afiqah Binti Lokman

2021

Supervisor: Dr Wan Mastura Bt Shaik Mohamed Mossadeq

Cassia alata (ringworm bush) is widely used in traditional medicine for its antifungal properties. This ornamental shrub often thrives in the humid conditions of the tropics. The applications of *Cassia alata* (*C. alata*) antifungal properties in veterinary medicine are systematically reviewed. This review was conducted in accordance to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) study protocol. A literature search was performed by identifying all journal articles that are related to the topic from 3 databases, namely Scopus, Science Direct and CAB Direct. A total of 313 articles published from 2000 to 2021 were selected. Abstracts and references of these articles were later exported to EndNote X9 for further scrutiny. Following the screening and eligibility process, 30 studies were found to be eligible, and all relevant data was tabulated using

Microsoft Office Excel 2016. A comprehensive review of the articles indicated that the most frequently used parts of the plant are the leaves, followed by flowers, roots, bark, and seeds. Organic extracts (e.g.: methanol and ethanol) were found to exhibit higher antifungal activity than other solvents. The antifungal effect of *C. alata* was mostly experimented on *Candida albicans* and *Trichophyton mentagrophytes*. The antifungal activity exhibited by *C. alata* extracts was equally effective against dermatophytes and yeasts. However, there were variations in the effectiveness of *C. alata* antifungal activities reported. The discrepancy in the effectiveness of this plant in the *in vitro* studies may be due to the variation in conditions of the study and/or the concentrations of crude extracts used. Therefore, determination of the exact phytochemical compound(s) responsible for the antifungal effect of *C. alata in vitro* is warranted. Nevertheless, the use of *C. alata* capped metal nanoparticles as an antifungal agent to overcome antimicrobial resistance seems promising at the moment.

Keywords: *Cassia alata*; Antifungal; Veterinary*; In vitro*

CHAPTER 1

INTRODUCTION

According to the World Health Organisation (WHO), utilisation of herbal medicine for natural remedy accounts for about 80% of the total population from developing countries (Oladeji et al., 2020). The pharmaceutical industry in general, play an important role in the development of novel drugs derived from medicinal plants. At present, it is estimated that almost 50% of contemporary drugs available in the market are derived from natural-based compounds (Meenupriya et al., 2014). The discovery of chemical compounds which are of therapeutic value from a plant is often attributed to its immense phytochemical components. However, the therapeutic value of the plants is strongly correlated to the bioactivity of the plant's secondary metabolites (Oladeji et al., 2016) such as tannins, flavonoids, alkaloids, phenolics and anthraquinones.

The search for novel antifungal agents from herbs and medicinal plants is needed as many microorganisms were found to exhibit resistance against the commercially available agents. It is known that effects of fungal infestation in human and animals has been greatly overlooked compared to the other aetiological agents, particularly due to the underestimation of fungal infections in animals and humans thus, creating a serious public health threat (Gnat et al., 2021). From the veterinary perspective, overstocking of animals in farms often lead to an unhygienic environment which intensifies the animal-human transmission of opportunistic pathogens (Moretti et al., 2013). The impact of climate change on the ubiquitous distribution of fungi also plays a significant role in the spread of this micro-organism; the effect of which is best observed in the immunocompromised patients (Gnat et al., 2021; Seyedmousavi et

al., 2015). *Sporothrix* spp. for instance thrives in the tropical and subtropical region known to be climate-sensitive, while dermatophytes activity is readily influenced by fluctuation of temperature and moisture content (Gnat et al., 2021).

Nevertheless, many antifungal agents that are designed for human use are also indicated for animals (Seyedmousavi et al., 2018). Regardless of its benefits for the treatment of fungal infections, the undesired side effects and antimicrobial resistance produced by these antifungal agents raises some concerns (Legaspi & Maramba-Lazarte, 2020). For instance, antifungal resistance by *Candida albicans* and *Cryptococcus neoformans* had been reported in humans, poultry and livestock (Bhanderi et al., 2009). This warrants the discovery of new antifungal agents with less side effects and medicinal herbs like *Cassia alata* has been hailed as a good candidate due to its pharmacologically active compounds (Legaspi & Maramba-Lazarte, 2020).

Senna alata or previously known as *Cassia alata* is an ornamental shrub that commonly thrives in the humid condition of the tropics. This plant first inhabited the Amazon Rainforest although it can now be found across Africa, Asia and South America (Oladeji et al., 2016). The plant is also known as candle bush due to its inflorescence. All parts of the plant can be used to treat various ailments such as constipation, eczema, stomach pain and even flu (Hennebelle et al., 2009). Nevertheless, this plant is famously known for its antifungal property, hence the common reference 'ringworm cassia' or 'ringworm bush' (Hennebelle et al., 2009).

The tannins, flavonoids, alkaloids, phenolics and anthraquinones found in *Cassia alata* serve as a protective mechanism against the destructive effects caused by microorganisms, invasive herbivores and insects (Oladeji et al., 2016). Interestingly, some of the compounds are responsible for the plant's pigment, taste

and odour (Cowan, 1999). Apart from the significant effect on the plant's vigour, these metabolites are responsible for its pharmacological activity, rendering *Cassia alata* as one of the popular medicinal plants in herbal medicine (Oladeji et al., 2016).

To date, there is no report on *Cassia alata* antifungal properties from the veterinary perspective. This systematic review aims to compile laboratory appraisals on the extraction methods used and the outcome from identified studies thus elucidating the bioactive compounds responsible for exhibiting the antifungal effects. The effect of *Cassia alata* antifungal properties on fungi of veterinary importance is also discussed.

CHAPTER 2

MATERIALS AND METHODS

As mentioned by Xiao and Watson (2017), in general, the outline for conducting literature reviews in systematic review can be sorted as follows: (1) formulate the research question, (2) plan or validate the review protocol, (3) conduct literature search, (4) perform screening process from pre-set inclusion criteria, (5) quality appraisal, (6) data extraction, (7) synthesis and analyse data and lastly, (8) document findings.

2.1 Review Protocol

The review protocol for this systematic review was guided by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) (Page et al., 2021). The research question was formulated using the PICO scheme (Population, Intervention, Control and Outcome). Precisely, "Population" stands for *Cassia alata*, "Intervention" is the setting or exposure condition in which for this context is *in vitro*, "Control" refers to antifungal and "Outcome" is the properties that make up the antifungal effects. Hence, the research question is 'What are the *Cassia alata in vitro* antifungal properties?'

2.2 Search Strategy

The literature search was conducted using 3 databases which were CAB Direct, Scopus and Science Direct. For each database, the search for related articles was performed using Boolean operators and phrase searching. Each keyword was also enriched appropriately. When the character and Boolean operators' limitations were encountered, few search strings were performed by limiting the total keywords included during the searching process through several attempts. During the literature search, the "Intervention" was omitted in all databases to capture more studies and relevant articles. The literature search took place on 06/09/2021 and was completed on 17/09/2021.

On CAB Direct, the keywords used were ("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-mycotic" OR "anti-dermatophyte") AND ("property" OR "properties" OR "activity" OR "activities" OR "characteristic" OR "characteristics" OR "effect" OR "effects"). Next, the search attempts were continued in Science Direct. In Science Direct, fewer Boolean connectors were required thus, several searching attempts were conducted. Overall, the keywords used were: ("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-dermatophyte" OR "anti-mycotic") AND ("property" OR "properties" OR "activity" or "activities" OR "effect" OR "effects" OR "characteristic" OR "characteristics"). A final article search was conducted in Scopus. Here, apart from Boolean operators, field codes and wildcards were utilised thus, the keywords were: TITLE-ABS-KEY ("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-mycotic" OR "anti-dermatophyte") AND ("activit*" OR "propert*" OR "characteristic*" OR "effect*"). 'Handpicking' method was used to select relevant articles in all databases utilised.

2.3 Study Selection

The compiled keywords used in all 3 databases are listed in Table 2.1. Abstract and references of identified literature were exported to EndNote X9 software and de-duplication was performed using the same software. All duplicates were later removed. Title and abstract screening were performed on the remaining articles and selected articles were brought to full text review for eligibility.

Table 2.1. Search string used in the selected databases

CAB Direct	("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-mycotic" OR "anti-dermatophyte") AND ("property" OR "properties" OR "activity" OR "activities" OR "characteristic" OR "characteristics" OR "effect" OR "effects")
Scopus	TITLE-ABS-KEY (("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-mycotic" OR "anti-dermatophyte") AND ("activit*" OR "propert*" OR "characteristic*" OR "effect*")
Science Direct	("*Cassia alata*" OR "*Senna alata*" OR "candle bush") AND ("antifungal" OR "anti-dermatophyte" OR "anti-mycotic") AND ("property" OR "properties" OR "activity" or "activities" OR "effect" OR "effects" OR "characteristic" OR "characteristics")

2.4 Inclusion and Exclusion Criteria

The inclusion criteria were the articles published must be within the year 2000-2021 and the abstract and full article must be written in English. The article must also include an *in vitro* antifungal study of *Cassia alata* extract with a clear methodology. No restriction was imposed for the type of antimicrobial assay used, the extraction method to extract *Cassia alata* or parts of the plant used. Also, the tested fungi with *Cassia alata* extract must be of the fungi species of veterinary importance. The result provided in the study can be either as Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC) or Minimum Fungicidal Concentration (MFC).

The exclusion criteria were studies not within the year 2000-2021 and studies with no access to full text or no full text available. Abstracts or full articles in languages other than English were excluded. Also, proceedings, books, surveys, clinical trials, reviews, systematic reviews, *in silico* and *in vivo* studies were rejected. Other irrelevant or unrelated articles such that involving phytochemical studies, toxicological studies, antifungal studies involving *Cassia alata* against plant pathogen, *in vitro* studies without assessing the antifungal activity of *Cassia* alata were not included. Microbial studies using *Cassia alata* extract without experiments on fungi (i.e.: bacteria) or fungi of veterinary importance were also disregarded.

2.5 Data Extraction

All relevant information (author, title, parts of the plant used, type of extract, type of antimicrobial assay, fungi tested and the ZOI or the MFC or the MIC value) of selected articles were extracted using Microsoft Office Excel 2016 sheet.

CHAPTER 3

RESULTS

3.1 Search Outcome

The flowchart of the systematic review which was guided by PRISMA is shown in Figure 3.1. From the effort of articles searching in all 3 databases, 313 articles were identified. A total of 38 duplicates were removed from the deduplication process and left with 275 articles for the title and abstract screening. After careful title and abstract screening, 226 articles were excluded and the remaining 49 articles were brought forward for full text review to be assessed for eligibility.

From the 49 articles selected for eligibility, two (2) articles were excluded due to no access to full text while one (1) article was published in a language other than English. Six (6) articles were excluded as the *Cassia alata* extract was tested on fungi of non-veterinary importance such as *Talaromyces marneffei* (Jeenkeawpieam et al., 2021), *Penicillium oxalicum* (Abas et al., 2020), *Geotrichum candidum* (Pieme et al., 2008), *Fusarium* sp., *Microsporum ferrugineum*, *Trichoderma* sp. along with mycotoxin producing fungi like *Aspergillus flavus* and *Aspergillus niger* (Ajayi et al., 2008; Ekwealor & Oyeka, 2015; Ekwealor et al., 2012). Two (2) articles that reported *in vitro* studies of *Cassia alata* extract on bacterial species which does not conform to the inclusion criteria (Hoffman et al., 2004; Khan et al., 2001) were excluded. Also, two (2) *in vitro* antifungal studies evaluating metal nanoparticles extracted from *Cassia alata* were rejected from inclusion as an antifungal activity through biofilm inhibition of tested fungi (*C. albicans*) was evaluated via optical density (Judan Cruz et al., 2021), while another study proceeded with highly-stabled leaf extract of *Cassia*

roxburghii since the silver nanoparticle extracted from aqueous leaf extract of *Cassia alata* exhibit instability during the silver nanoparticle phytosynthesis (Balashanmugam et al., 2016). One (1) silver nanoparticle study by Thiagamani et al. (2019) evaluated the antimicrobial activity of banana peel powder and hence, was rejected.

Some studies performed antimicrobial activity using several types of extracts from various parts of *Cassia alata* but the yielded extracts failed to exhibit any activity or were inactive to the tested fungi. Duraipandiyan et al. (2006) tested 2 forms of extracts, hexane and methanol extracts of 18 plants by cold percolation method and appraised each plant's antimicrobial characteristics via disc diffusion method and revealed methanol extracts had impressive activity against tested microbes. Nonetheless, *Cassia alata* did not exhibit the same activity on the only tested fungi (*C. albicans*) despite a significant inhibitory activity of several bacteria. Similar circumstances were observed by Salihu et al. (2012); Sukumar and James (2012) and Idu et al. (2007). A study evaluating antifungal action of *Cassia alata* by do Nascimento et al. (2020) was >3000.0 µg/ml for *C. albicans* and was considered inactive by the author. These studies were not included in the study. The final number of studies included was 30, as shown in Figure 3.1.

3.2 Results of The Included Studies

The compiled results and characteristics of each study included are listed in Table 3.1. Figure 3.2 represents the most frequently used part from *Cassia alata* for the antifungal assessment, Figure 3.3 is the type of extract used for *Cassia alata* extraction by the number of articles, Figure 3.4 is the number of studies conducted on yeast and yeast-like organism and Figure 3.5 is the number of studies conducted on dermatophytes.

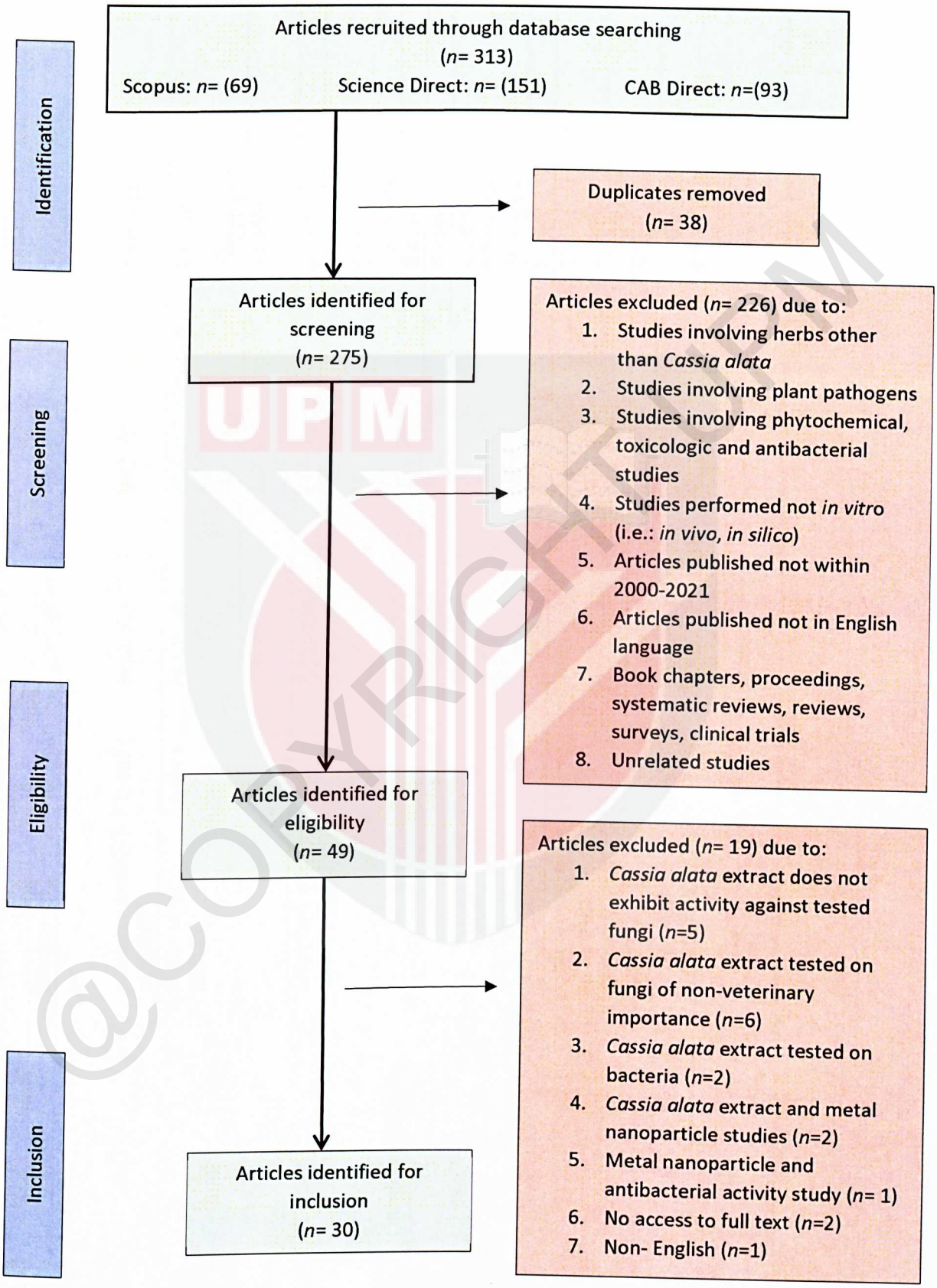

Figure 3.1. Flowchart of the article search process as guided and described by PRISMA

Table 3.1. Summary of studies on *C. alata* extract tested on multiple fungi species

Fungi tested	Study	Plant part	Types of extract	Antifungal assay	ZOI / MIC / MFC
Yeast					
Candida albicans	Abubacker et al. (2008)	Flower	Aqueous	MIC, BD	15 mg/ml
	Alalor et al. (2012)	Leaves	Aqueous	ZOI at 200 mg/ml, AWD MIC, AD	22 mm 160 mg/ml
			Methanol	ZOI at 200 mg/ml, AWD	16 mm
	Doughari and Okafor (2007)	Root	Aqueous	ZOI, AWD	6 mm
			Methanol	ZOI, AWD MIC, BD MFC	8 mm 50 mg/ml 50 mg/ml
			Acetone	ZOI, AWD	8 mm
		Leaves	Aqueous	ZOI, AWD	6 mm
			Methanol	ZOI, AWD MIC, BD MFC	8 mm 50 mg/ml 50 mg/ml
			Acetone	ZOI, AWD	6 mm
	Gbadamosi et al. (2012)	Leaves	Methanol	ZOI, AWD MIC, BD	25.0 ± 10.0 mm 5 mg/ml
	Makinde et al. (2007)		Aqueous	ZOI, DD	10-20 mm
			Methanol	ZOI, DD	10-20 mm
	Okwu and Nnamdi (2011)	Seeds	Ethanol extract transformed into cannabinoid dronabiol alkaloid	ZOI, DD MIC, BD	12.0 ± 0.20 mm 6.5 – 50 mg/ml

Candida albicans	Owoyale et al. (2005)	Leaves	Petroleum ether	ZOI, AWD	2.1 mm
			Ethanol	ZOI, AWD	2 mm
			Methanol	ZOI, AWD	2 mm
			Flavonoid glycoside by Thin Layer Chromatography (TLC)	MIC, AD	860 µg/ml
	Parbin et al. (2019)	Leaves	Methanol	ZOI, AWD	23.66 ± 0.33 mm
			Bioactive compound (PF 1), contains alcohol and ketone, obtained from methanol extract incorporated into an herbal gel	ZOI, AWD MIC, BD	31.66 ± 0.65 mm 120 µg/ml
	Somchit et al. (2003)	Bark	Ethanol	ZOI, DD	10.2 ± 0.1 mm
			Aqueous	ZOI, DD	12.3 ± 0.7 mm
	Timothy et al. (2012)	Leaves	Aqueous	ZOI, AWD, MIC, extrapolation from ZOI	30.00 ± 0.78 mm 26.9 mg/ml
			Ethanol	ZOI, AWD MIC, extrapolation from ZOI	36.00 ± 0.81 mm 5.6 mg/ml
	Villaseñor et al. (2002)	Leaves	Hexane	ZOI, AWD	12 mm
			Ethyl acetate	ZOI, AWD	15 mm

	Wuthi-udomlert et al. (2003)	Leaves	Ethanol	ZOI, AWD	18.8 mm
			Aqueous	ZOI, AWD	14.2 mm
			Crude anthraquinone	ZOI, AWD	10.7 mm
	Adongbede and Wisdom (2013)	Leaves	Ethanol	ZOI, DD	20 mm
			Methanol	ZOI, DD	20.33 mm
			n-hexane	ZOI, DD	10.33 mm
	Bharti et al. (2013)	Leaves	Methanol	ZOI, DD	8.00 mm
	Nazmul et al. (2011)	Plant extract	Methanol	ZOI, DD	8.00 mm
	Ontong et al. (2019)	Bark	Biosynthesis of silver nanoparticles from bark extract	ZOI, AWD MIC, BD MFC	11.37 ± 0.17 mm 31.25 µg/ml 62.50 µg/ml
Candida albicans	Zanna et al. (2021)	Root	98% methanol	ZOI, AWD MIC, BD	39.55 ± 1.2 mm 312.5 µg/ml
	Cheeptham and Towers (2002)	Flower, leaves	Ethanol	ZOI, light-mediated ultraviolet (UV) antifungal activities at 5W / m^2	8-12 mm
	Majekodunmi and Essien (2014)	Leaves	Methanol	ZOI, DD	6.3 ± 0.5 mm
Candida krusei	Adongbede and Wisdom (2013)	Leaves	Ethanol	ZOI, DD	20.00 mm
			Methanol	ZOI, DD	20.33 mm
			n-hexane	ZOI, DD	13.67 mm
			Chloroform	ZOI, DD	8.67 mm
			Aqueous	ZOI, DD	9.00 mm
		Root	Aqueous	ZOI, AWD	6 mm

Cryptococcus neoformans	Doughari and Okafor (2007)		Methanol	ZOI, AWD MIC, BD MFC	8 mm 12 mg/ml 12 mg/ml
			Acetone	ZOI, AWD	8 mm
		Leaves	Aqueous	ZOI, AWD	6 mm
			Methanol	ZOI, AWD MIC, BD MFC	8 mm 25 mg/ml 25 mg/ml
			Acetone	ZOI, AWD	6 mm
Malassezia furfur	Prabhu et al. (2020)	Flower	Chloroform	ZOI, AWD	4.7 ± 0.01 mm
			Ethanol	ZOI, AWD	7.7 ± 0.03 mm
Dermatophytes					
Trichophyton mentagrophytes	Adejumo and Bamidele (2009)	Leaves	Aqueous	Mycelial growth inhibition (%)	71% at 0.005 mg/ml
			Ethanol	Mycelial growth inhibition (%)	100% at 0.002 mg/ml
	Alalor et al. (2012)	Leaves	Aqueous	ZOI at 200 mg/ml, AWD MIC, AD	23 mm 80 mg/ml
			Methanol	ZOI at 200 mg/ml, AWD	20 mm
	Chellappandian et al. (2018)	Leaves	Ethanol	MIC, BD MFC	62.5 µg/ml 250 µg/ml
	Makinde et al. (2007)	Leaves	Aqueous	ZOI, DD	10-20 mm
			Methanol	ZOI, DD	20-30 mm
	Parbin et al. (2019)	Leaves	Methanol	ZOI, AWD	21.33 ± 0.33 mm
	Sadeghi-Nejad and Azish (2013)	Leaves	Ethanol	ZOI, AWD MIC, BD	20 mm 20 mg/ml

	Sakunpak et al. (2009)	Leaves	High yield anthraquinone via purification	MIC, AD	62.5 µg/ml
	Sule et al. (2010)	Leaves	Ethanol	ZOI, AWD MIC, BD MFC	17 mm 5 mg/ml 5 mg/ml
	Sule et al. (2011)	Stem bark	Ethanol	ZOI, AWD MIC, BD MFC	17 mm 5 mg/ml 5 mg/ml
Trichophyton mentagrophytes	Timothy et al. (2012)	Leaves	Aqueous	ZOI, AWD MIC, extrapolation from ZOI	35.00 ± 0.58 mm 27.8 mg/ml
			Ethanol	ZOI, AWD MIC, extrapolation from ZOI	30.00 ± 0.58 mm 9.8 mg/ml
	Villaseñor et al. (2002)	Leaves	Hexane	ZOI, AWD	14 mm
			Ethyl acetate	ZOI, AWD	16 mm
			Chloroform	ZOI, AWD	22 mm
	Nantachit (2009)	Leaves	Ethanol	ZOI, AWD	35 mm
			10% methanol	ZOI, AWD MIC, AD	37 mm 15 mg/ml
	Nazmul et al. (2011)	Plant extract	Methanol	ZOI, DD	10 mm
	Sujatha et al. (2019)	Leaves	Ethanol	ZOI, AWD MIC, BD MFC	7.27 ± 0.15 mm 63.00 mg/ml 75.00 mg/ml

	Vaijayanthimala et al. (2004)	Leaves	Aqueous	MIC, BD MFC	18.7 mg/ml 18.7 mg/ml
			Ethanol	MIC, BD MFC	2.3 mg/ml 2.3 mg/ml
	Duraipandiyan and Ignacimuthu (2011)	Flower	Hexane	MIC, BD	0.5 mg/ml
			Ethyl acetate	MIC, BD	1.0 mg/ml
Microsporum canis	Makinde et al. (2007)	Leaves	Aqueous Methanol	ZOI, DD ZOI, DD	10-20 mm 20-30 mm
	Parbin et al. (2019)	Leaves	Methanol	ZOI, AWD	21.66 ± 0.33 mm
	Sule et al. (2010)	Leaves	Ethanol	ZOI, AWD MIC, BD MFC	12.05 mm 5 mg/ml 5 mg/ml
	Timothy et al. (2012)	Leaves	Aqueous	ZOI, AWD MIC, extrapolation from ZOI	32.00 ± 0.78 mm 30.3 mg/ml
			Ethanol	ZOI, AWD MIC, extrapolation from ZOI	30.00 ± 0.48 mm 12.6 mg/ml
	Nazmul et al. (2011)	Plant extract	Methanol	ZOI, DD	12 mm
	Sujatha et al. (2019)	Leaves	Ethanol	ZOI, AWD MIC, BD	10.67 ± 0.44 mm 95 µg/ml
Trichophyton verrucosum	Sule et al. (2010)	Leaves	Ethanol	ZOI, AWD MIC, BD MFC	16.5 mm 5 mg/ml 5 mg/ml
	Sule et al. (2011)	Stem bark	Ethanol	ZOI, AWD MIC, BD MFC	15 mm 5 mg/ml 5 mg/ml

Microsporum gypseum	Chellappandian et al. (2018)	Leaves	Ethanol	MIC, BD MFC	62.5 µg/ml 250 µg/ml
	Sakunpak et al. (2009)	Leaves	High yield anthraquinone via purification	MIC, AD	250 µg/ml

ZOI: zone of inhibition; MIC: minimum inhibitory concentration, MFC: minimum fungicidal concentration; AWD: agar well diffusion; DD: disc diffusion; BD: broth dilution; AD: agar dilution.

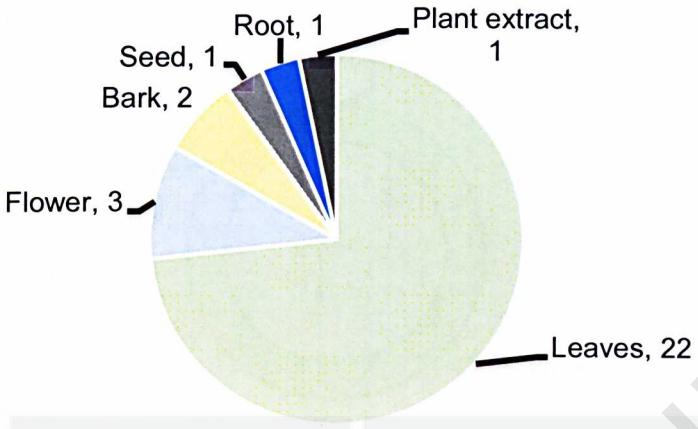

Figure 3.2. The number of antifungal studies conducted using various parts from *Cassia alata.*

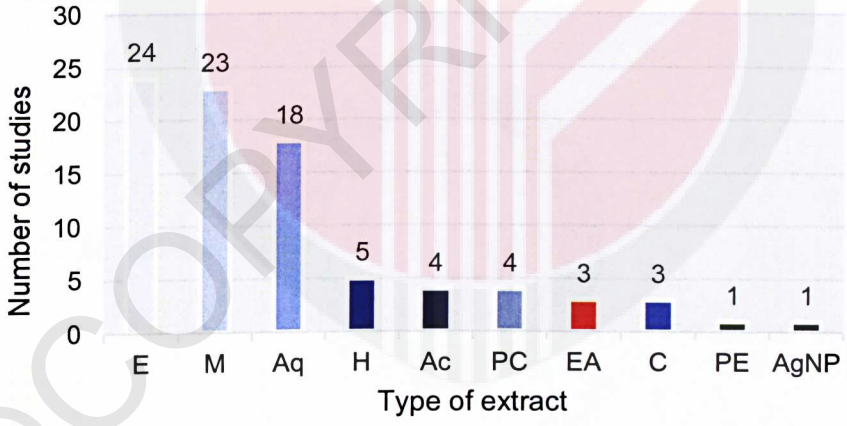

Figure 3.3. The type of extracts used for *Cassia alata* extraction by the number of articles. E: ethanol; M: methanol; Aq: Aqueous; H: hexane, Ac: acetone; PC: phytochemicals; EA: ethyl acetate; C: chloroform; PE: plant extract; AgNP: silver nanoparticle

Yeast and yeast-like fungi

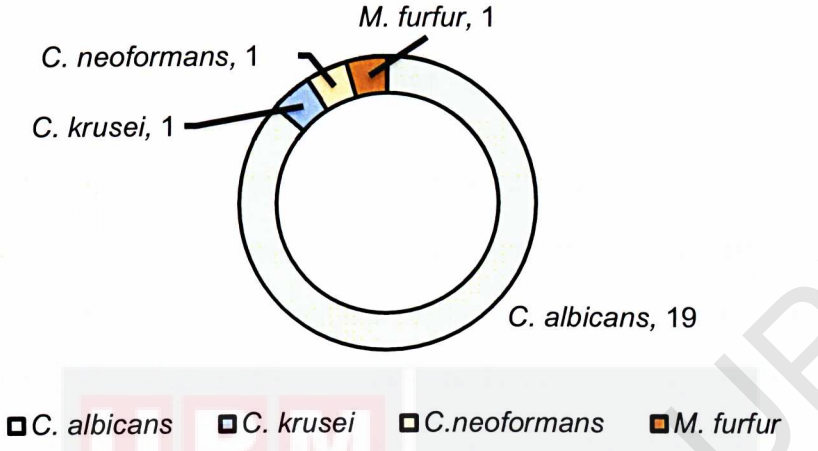

Figure 3.4. The number of studies conducted on yeast and yeast-like fungi species

Dermatophytes

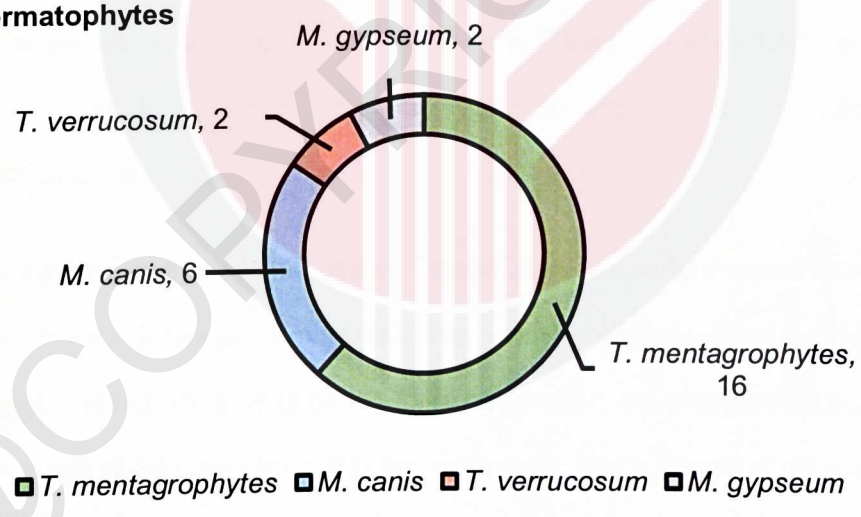

Figure 3.5. The number of studies conducted on dermatophytes species

CHAPTER 4

DISCUSSION

A total of 30 *in vitro* studies evaluated the antifungal activities of *Cassia alata* emphasizing yeast and dermatophyte species of veterinary importance. Different parts of the plant were used however, the most frequently used parts are the leaves and followed by flowers, roots, barks and seeds. From the findings, it was observed that the most frequently used extraction solvents are ethanol, followed by methanol and aqueous extraction. Evaluation of the inhibitory activity via ZOI was frequently conducted through agar well diffusion (AWD) or disc diffusion (DD). In addition, some studies also tested the antifungal potency of *Cassia alata* extract via MFC and MIC through broth dilution (BD) and agar diffusion (AD). MIC is defined as the minimum extract concentration that visibly inhibit the growth of the fungi while MFC constitutes the lowest concentration from MIC that prevents fungi growth on solid media (Vaijayanthimala et al., 2004).

In addition to the contribution of *Cassia alata* secondary metabolites in the antifungal efficacy of the plant extracts, other forms of enhancement methods may also contribute to the effect. In targeting fungi obliteration, the pharmacological action target site ought to be highlighted. It is known that the major sterols of the eukaryotic fungi cell membrane are ergosterol while in the mammalian cell membrane, the major sterols comprise of cholesterol (Foy & Trepanier, 2010). Ergosterol quantifications not only can act as a biomarker, but it also facilitates fungi identification and signals fungi growth impediments (Chellappandian et al., 2018).

The three most common pathogenic dermatophytes in small animals are *M. canis*, *Trichophyton* spp. and *M. gypseum* (Moriello et al., 2017). Dermatophytosis in

animals can be manifested primarily as skin lesions that are confined to the stratum corneum of the skin and keratinised layer of hair and nails (Adebiyi & Oluwayelu, 2018). It is the most common skin ailments reported in small animal clinics (Mattei et al., 2014). In rabbits, dermatophytosis is almost always caused by *T. mentagrophytes* var. *mentagrophytes* while ruminants is the reservoir for *T. verrucosum* (Mattei et al., 2014; Moretti et al., 2013). Dermatophytes are not one of the skin's normal floras, render their infection in either animals or humans unnatural (Gnat et al., 2021).

Chellappandian et al. (2018) conducted a fungal cell toxicity study targeting the ergosterol of dermatophytes (*T. mentagrophytes*, *T. rubrum*, *T. simii, T. tonsurans* and *Microsporum gypseum*) using ethanolic crude extract of 18 medicinal herbs from different parts of the plants. After fungal cells treated with the extract were incubated, ergosterol quantification and identification by spectrophotometry were conducted. Isolation of ergosterol was done via saponification whereas non-saponifiable lipid was done via hexane extraction. It was revealed that *Cassia alata* controls ergosterol biosynthesis at 21.44 % that significantly inhibit the fungal activity of the tested isolates (Chellappandian et al., 2018). Nonetheless, the remarkable result could potentially serve *Cassia alata* to be exploited as an antifungal drug in the future.

Candidiasis can invade the skin, mucosa of the urogenital or gastrointestinal tract and in birds, candidiasis commonly manifested as oral candidiasis or gastrointestinal candidiasis (Seyedmousavi et al., 2018). In poultry farms, candidiasis is one of the most important fungal diseases apart from aspergillosis (Mohammed & Abdel-Latef, 2021). Meanwhile, systemic infections in dogs and cats are rare but accidental inoculation of *Candida* into the peritoneal cavity or into deeper tissue during surgery and trauma leading to overt inflammatory reaction has been reported (Seyedmousavi et al., 2018). Besides, it was documented that cutaneous candidiasis in small animals is rather frequent, particularly in dogs (Seyedmousavi et al., 2018). Other fungi worth mentioning are from the genus *Cryptococcus* and *Malassezia*. *Cryptococcus neoformans* chiefly infect immunocompromised patients (Seyedmousavi et al., 2018). In dogs and cats, cryptococcosis disseminated infection or inoculation of the agent in the upper or lower respiratory tract frequently involved, with central nervous system (CNS) manifestation is commonly reported in dogs (Seyedmousavi et al., 2018).

The genus *Malassezia* is one of the inhabitants of the skin as microflora in mammals (Bond et al., 2020). For example, in healthy dogs, *Malassezia pachydermatis* is known as commensals in ear canals, on the skin and mucosal surfaces (Bond et al., 2020; Peano et al., 2020). Most common clinical reports were noted on *M. pachydermatis* in dogs but other species such as *M. furfur* causing otitis and dermatitis have been occasionally mentioned as well (Bond et al., 2020). Recently, a study noted that the whole genus of *Malassezia* are lipid-dependent from a genomic sequencing study which had revealed a gene that encodes the fatty acid synthase is absent in all *Malassezia* species (Peano et al., 2020). This opens up to a discovery that certain fatty acids may have an inhibitory activity on *Malassezia* spp.

despite the whole genus being lipophilic in nature but can still thrive without entirely depend on lipids (Peano et al., 2020).

Prabhu et al. (2020) assessed fatty acids and phytochemicals from the flowers of *Cassia alata* and *Cassia auriculata* to investigate the potential anti-dandruff activity from the compounds yielded. The antidandruff compounds were harvested from the flower chloroform and ethanolic extracts via gas chromatography-mass spectrophotometry (GC-MS). From the preliminary *in vitro* assessment, ethanolic extracts elicit higher inhibitory activity against *M. furfur* than chloroform extract at a concentration-dependent manner. Many bioactive compounds were obtained from the GC-MS analysis, one of them is citronellol (terpenoids) that is present in *Cassia alata* ethanolic extract. Resorcinol (flavonoids) was also obtained and this compound is known to have anti-dermatophytic and keratolytic activities. Lipid compounds comprised in the form of fatty acids and fatty alcohols were responsible for the anti-malassezial activity as well (Prabhu et al., 2020).

From the study conducted by Prabhu et al. (2020), further purification is needed to identify a particular phytochemical to establish a novel, plant-based antidandruff formulation. Besides, the inhibitory mechanism elicited by medium-chain triglycerides and medium-chain free fatty acids by Mayser (2015) was said to be toxic against *Malassezia* spp. apart from the similar activity derived from lipids in the study by Prabhu et al. (2020).

A nanoparticle (NP) study performed by Ontong et al. (2019) evaluated the antimicrobial activity of *Cassia alata* and the biosynthesis of silver nanoparticles (AgNPs) using the bark extract against *C. albicans*, *E. coli*, *K. pnemoniae*, *P. aeruginosa* and *A. baumannii*. The antimicrobial activity of AgNPs against tested fungi at 50 µg/well was enhanced with ZOI of 11.37 ± 0.17 mm and MFC of 62.5 µg/ml, a remarkable potency exhibited by the NPs compared to nil activity shown by the aqueous extract against all tested microorganisms (Ontong et al., 2019). The antimicrobial activity of the AgNPs was due to the NPs reaction with the transporter protein, enzymes, nucleic acids and strong affinity to the hyphae membrane which eventually brings to the cytotoxicity of the fungi (Ontong et al., 2019). Under scanning microscope, microorganisms exposed to the nanoparticles exhibit corrugated, roughened and atrophied morphology due to potassium ion leakage (Ontong et al., 2019). Since potassium constitutes the main ions in the organisms' cells, AgNPs accumulation on the cell wall surface will cause hoard leakage of potassium ions due to membrane electrical potential exhaustion and formation of pores on the membrane which brings about the breakdown of membrane integrity, ultimately overt cell content leakage and cell death (Balashanmugam et al., 2016; Ontong et al., 2019).

Nanoparticle studies promote a breakthrough to the development of nano antimicrobial agents which are safe, eco-friendly and non-toxic owing to plants' massive range of phytochemicals that can act as stabilizing and reducing agents during nanoparticles synthesis (Happy et al., 2019). This technology has been applied in the cosmetic, food and pharmaceutical industries and is foreseen to contribute as a promising medium to combat the emergence of antimicrobial resistance (Ali et al., 2016; Happy et al., 2019).

Some of the chemicals said to be responsible for the antifungal activity are quinones, phenols, tannins, flavonoids and glycosides (Vaijayanthimala et al., 2004). Preliminary phytochemical screening performed by Zanna et al. (2021) on methanolic root extract of *Cassia alata* attained alkaloids, anthraquinone, flavonoids and tannins. Idu et al. (2007) conducted a similar preliminary screening on *Cassia alata* flower revealed major phytochemical groups from alkaloid, anthraquinone, flavonoid, tannins, saponins and glycosides. In addition, arrays of phytochemicals documented are anthraquinones (alatinone and alatonal), phenolics (rhein, emodin, kaempferol, glycosides and aloe-emodin), fatty acids (palmitic, oleic and linoleic acids), terpenoids (sitosterol and stigmasterol) and steroids (Oladeji et al., 2020). Nevertheless, not all phytochemicals are medicinal in nature although identification of each compound is equally important (Adelowo & Oladeji, 2017).

Substantial studies conducting phytochemical screening in medicinal plants have isolated about 200,000 phytochemicals (Oladeji et al., 2020). It has been discussed that bioactive components in medicinal plants act synergistically as some authors quoted that the mechanism behind a plant's biological activities is chemical compounds that do not act as a single entity (Adelowo & Oladeji, 2017; Oladeji et al., 2020; Zanna et al., 2021). Despite a vast number of compounds that have been identified, few clarifications made on the actual properties of the compounds (Martins et al., 2015).

Okwu and Nnamdi (2011) isolated a phytochemical compound from the ethanolic seed extract of *Cassia alata* and later analysed the antifungal activity of the metabolite against *C. albicans*, *A. niger*, *S. aureus*, *E. coli* and *P. aeruginosa*. The elucidation process through nuclear magnetic resonance (NMR), spectroscopy coupled with infrared (IR) and mass spectrum (MS) spectral data were used to obtain

the chemical compound. The isolated compound was identified as cannabinoid alkaloid (4-butylamine 10-methyl-6-hydroxy cannabinoid alkaloid) or compound 1. The compound exhibited a remarkable antimicrobial effect against the tested microbes and halted *Candida* cells proliferation. Okwu and Nnamdi (2011) explained the possible mechanism responsible for the inhibitory action could potentially be rooted in the phenolic compound that caused impairment of the enzymatic system of the fungi cell membrane, distorts the transfer of energy to the cell that eventually interfere the cell membrane's integrity and other essential processes. An identical interaction of phenolic compounds against fungi by affecting the cell membrane lipid bilayer and consequently alter the energy production, respiratory chain and membrane permeability was described by Martins et al. (2015). Another possible mechanism described by Okwu and Nnamdi (2011) was a compound ability to scavenge microorganisms attributed to the phenolic ring and diminished enzymes in the organism contributed by alkaloid and ether components. Okwu and Nnamdi (2011) further clarified that the destruction of fungi due to hyphae swelling followed by plasma leakage led to weaken cell wall and distorts hyphae fusion owing to the compound mentioned latter.

Sakunpak et al. (2009) purified *Cassia alata* leaf extract via silica gel vacuum chromatography and obtained a high-yielding anthraquinone. This method proved to exhibit the highest antifungal activity against tested dermatophytes (*T. mentagrophytes*, *T. rubrum*, and *M. gypseum*) at MIC values between 15.62-250 µg/ml. Sakunpak et al. (2009) also documented that the greatest antifungal activity was possessed by aloe-emodin against *T. rubrum* at MIC value of 0.98 µg/ml. In addition, complete inhibition of dermatophytes were displayed by rhein and emodin at 1.95-1000 and 31.25-1000 µg/ml, respectively (Sakunpak et al., 2009). *Cassia*

species are rich in anthraquinones and researchers have been isolated more than 100 anthraquinones derivatives, known for their use in countless fungal infections (Khurm et al., 2021). Being one of the anthraquinone components, it was justifiable that aloe-emodin and rhein were the major constituents responsible for the antifungal activity in the leaves of *Cassia alata* (Gritsanapan & Mangmeesri, 2009; Phongpaichit et al., 2004).

Many factors seem to play in tandem in determining the antifungal activity and phytochemical constituents of *Cassia alata*, hence the discrepancies of the secondary metabolites evaluated (Oladeji et al., 2020). Geographical location, climatic factors, age of the herb, and the germination conditions to name a few, but researchers believe the focus on phytochemical studies should be shifted to other parts of the herb, namely seeds, roots and barks since most phytochemical studies accentuated on the leaves, flowers and stems (Fatmawati et al., 2020; Oladeji et al., 2020). A standardised laboratory methodology for *in vitro* appraisal of secondary metabolites in medicinal herbs may help to overcome this matter to reproduce a comparative result in evaluating the antifungal potential of medicinal plants (Waller et al., 2017).

Adejumo and Bamidele (2009) tested aqueous and ethanolic *Cassia alata* leaves extract on clinical isolates of *T. mentagrophytes* and *T. rubrum* and discovered that ethanolic leaves extract had better mycelial inhibitory activity against *T. mentagrophytes* (71% at 0.005 mg/ml) and 100% inhibitory activity against *T. ruburm* at 0.002 mg/ml than aqueous extract. Another study by Vaijayanthimala et al. (2004) performed an *in vitro* experiment and evaluated anti-dermatophytic activity on 23 South Indian medicinal herbs against *T. mentagrophytes* and *T. rubrum* and approved that *Cassia alata* ethanolic leaves extract was better than aqueous extract. The ethanolic leaves extract had an MFC value of 2.3 mg/ml which was lower than

reported by Ibrahim and Osman (1995) at 125 mg/ml. It was suggested by Nantachit (2009) and Adejumo and Bamidele (2009) that ethanol may be a preferred choice of solvent for extraction.

Zanna et al. (2021) examined seven medicinal herbs for an antifungal assessment and reported that methanolic root extract of *Cassia alata* is among the three most potent extracts apart from the leaf methanolic extract of *O. gratissimum* and *B. dalzielii* that exhibited antifungal activity against *C. albicans* at a dose-dependent manner. These findings are parallel with Makinde et al. (2007) and Doughari and Okafor (2007). Doughari and Okafor (2007) appraised root and leaves of *Cassia alata* using aqueous, acetone and methanol for extraction. They tested all of the crude extracts against *A. flavus*, *A. niger*, *C. albicans* and *C. neoformans*. They concluded that the highest activity displayed by the roots and leaves methanolic extract, having the MIC and MFC range of 25 – 100 mg/ml compared to the control which was nystatin and amphotericin B at the range of 12-100 mg/ml. From this study, the most susceptible fungi against the extracts were *C. albicans* and *C. neoformans* (Doughari & Okafor, 2007). Doughari and Okafor (2007) concluded that highest activity against the tested fungi was displayed by the organic crude extracts than the aqueous extract hence highlighted different solubility possessed by different solvents may influence phytochemicals solubility capacities which explain the inconsistent activities manifested from different extracts employed. A contrast findings by Alalor et al. (2012) evaluated aqueous and methanolic leaves extract and reported that both extracts were potentially inhibitory with aqueous extract displayed slightly higher ZOI at 200 mg/ml against *C. albicans* and *T. mentagrophytes* than methanolic extract at a concentration-dependent manner. This highlights that the higher the extract's concentration, the higher the antifungal activity exhibited by the plant extract.

A similar test conducted by Nazmul et al. (2011) found that *Cassia alata* methanolic plant extracts had significant inhibitory activity against *M. canis* at ZOI of 12.0 mm than 8.0 mm for *C.albicans* and 10.0 mm for *T.mentagrophytes*, considered a significant antifungal activity of the plant extracts at a cut-off point of 10 mm. Another study evaluated the antimicrobial activity of *C.alata* crude flower extracts by Idu et al. (2007) using 4 different solvents which were methanol, chloroform, distilled water, and petroleum ether. However, none of the extracts possessed sensitivity against *C.albicans* albeit activity exhibited by methanol flower extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* at 500 µg/ml (Idu et al., 2007). On a side note, Adelowo and Oladeji (2017) recommended the use of solvents with high polarity as it bears greater secondary metabolites and concluded that methanol possessed higher capability in exerting antimicrobial effects than other solvents.

The distinction between plant's biological activity and the degree of inhibitory activity is influenced by the type of solvent, solvents' polarities utilised and parts of the plants used during the extraction thus accentuating the importance of using the correct solvent when extracting medicinal herbs (Adongbede & Wisdom, 2013; Zanna et al., 2021).

CHAPTER 5

CONCLUSION

The antifungal activity shown by *Cassia alata* through various *in vitro* methodology and several extraction methods against pathogenic fungi in the present review validates its use in treating ringworm. Still, further evaluation can be done on animal model since most recruited studies in the present investigation encompass *in vitro* antifungal evaluations. Some setbacks faced throughout conducting this review was the majority of the studies included were tested on fungi of human importance hence, full appraisal of antifungal effectiveness of *C. alata* extract tested against fungal diseases in animals was not achieved. From the present studies, organic extracts (*e.g.*. methanol and ethanol) exhibit better performance at inhibiting fungi when tested *in vitro* than other extracts since organic extract acquire higher polarity to harvest greater phytochemical compounds. The most frequently studied component from the plant was the leaves but other parts of the herb especially roots, bark and seeds require further analysis as well. Result differences obtained from some studies created discrepancies that signify a standardised laboratory methodology may help to overcome the problem and able to reproduce stable comparative results. Ultimately, it is crucial to identify and characterise the biological chemical compounds responsible for the antifungal effect to elucidate the mechanism of a particular chemical component responsible for the antifungal activity.

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