REVIEW ARTICLE

The Phytochemical Content and the *In vitro* Antifungal Properties of *Senna alata* (Linn.) Roxb.: A Review

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ABSTRACT

Infections caused by invasive fungal species continue to rise due to various contributing factors including the changes in the environmental and weather conditions, lifestyle, the occurrence of natural disasters, and the weakened human immune system. Even though synthetic drugs effectively cure fungal diseases, their unwanted side effects, as well as the rapid rise in resistance, have compelled researchers to develop new antifungal agents. Several medicinal plants are folklorically known to have antifungal activities. Among the traditionally used antifungal herbal plants is *Senna alata*, commonly known as akapulko and ringworm bush. In the current review, phytochemical analysis and numerous non-clinical studies on akapulko have been performed and confirmed its activity against several fungi pathogenic to humans. Anthraquinone compounds seemed to be the major phytochemicals responsible for its antifungal activity. In the Philippines, clinical trials have also confirmed its utility as a topical agent in treating cutaneous fungal infections. Research gaps that need to be addressed include the determination of the exact molecular mechanisms of their fungal killing action.

Key Words: antifungal, Senna alata, akapulko

INTRODUCTION

Diseases and infections caused by invasive fungal species have continued to rise and emerge. Changes in the environment, different lifestyles, the shifting weather conditions, ability of the fungi to alter its virulence factors, compromised immune system of the host, seasonal migration, and occurrence of natural disasters were cited as the major contributing factors for fungal invasion.[1,2] Majority of the reports for infections were due to newly discovered fungi and even re-emerging fungal pathogens that can reconstruct their virulence factors.[1] In the Philippines, the warm and humid tropical climate, and the age, type of occupation, genetic susceptibility, and immune sensitivity of the Filipinos favor fungal infection pervasiveness. The most common fungal infection cases in the country include pityriasis versicolor (25.34%), tinea corporis (22.63%), tinea cruris (16.7%), and tinea pedis (16.38%)[3]; in 2017, 1.9% of the population suffers from serious fungal infections.[4]

Countering the effect and spread of fungal infections, commercially available antifungal drugs such as the polyenes (amphotericin B) and azoles (fluconazole, itraconazole, voriconazole, and posaconazole) disrupt fungal cell wall stability and destroy the pathogen's homeostasis causing osmotic stress.[5] Amphotericin B is the major choice in treating fungal cystitis, peritonitis, dermatoses, and

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intraocular infections and is effective against *Candida albicans*[5,6,7] by binding to the plasma membrane component ergosterol causing cell leakage and death. On the other hand, azoles, a group of broad-spectrum fungistatic agents, inhibit the enzymes for ergosterol synthesis, an important enzyme for fungal cell wall biosynthesis.[5,8] While deemed effective, these synthetic drugs have features that limit their use. The nephrotoxicity caused by Amphotericin B[9,10] is a well-known side effect; in the use of azoles, it requires cytochrome P450-induced oxidative metabolism to be eliminated from the body.[8,11] Other reported side effects of azoles were nausea, vomiting, headache, abdominal pain, skin infections, occasional increase in liver serum enzymes, pruritus, hepatotoxicity, and gynecomastia.[12]

Considering that these synthesized drugs have its share of negative side effects and are expensive, and that fungi continually develop resistance against them, the search for alternative sources of therapy is necessary. Medicinal plants have long been used as a source of bioactive compounds for modern medications. Those with folkloric use as an antifungal aid were most likely candidates for the discovery of treatments for fungal infections. Among the plants traditionally used for fungal infection is *Senna alata* (Linn.) Roxb. (synonym *Cassia alata* Linn.) of the Family Fabaceae/Leguminosae), more commonly known as akapulko, ringworm bush, Christmas candle, candle brush, or calabra brush.[13,14]

Akapulko is an erect perennial shrub, originally cultivated as an ornament[15,16] native to Southeast Asia (Japan and Indonesia), Africa (Ghana, Nigeria), Northern Australia, and Latin America (Mexico).[17,18,19] It is traditionally used as a laxative, purgative, and as treatment to skin problems.[14,16,20] The commonly used parts were the leaves, bark, stem, root, pod, and seeds[21]; however, majority of the documented reports used the leaves and the roots. The leaves were said to be sudorific, diuretic and purgative,[22,23] and its decoction can treat bronchitis and asthma.[23] On the other hand, the roots were traditionally used as a pain reliever for dysmenorrhea, stomach pain during pregnancy, dysentery, convulsion, heart failure, edema, jaundice, and paralysis in tropical Africa.[24]

Phytochemicals Responsible for its Antifungal Action

Phytochemicals are biologically active compounds produced by plants, dedicated for their survival and adaptation. There are five general classifications of phytochemicals: carbohydrates, lipids, terpenoids, phenolic acids, and alkaloids and other nitrogen-containing metabolites.[25] In most cases, the type and concentration of the phytochemicals present in a specific plant is a product of different factors such as the type of soil where it was planted, cultivation method, type of fertilizer used, the weather condition, season, and even with the time of the day. In addition, the amount of total flavonoids, phenolics, flavonols, condensed tannins, and carotenoids that can be extracted from a plant is affected by the type, concentration, pH, polarity, and temperature of solvent used for extraction, plant part used, age of the plant, genotype or variety of the plant, and the type of extraction method performed (e.g. maceration, homogenization, etc.).[26,27,28] A recent study showed that phytochemical concentration, specifically of the glucosinolates and the antioxidant activity of the two genotypes of cauliflower changed through-out the three-year study.[29] The scarce rainfall during the third year of their study showed increased glucosinolates and decreased antioxidant activity. In another study, the increasing amount of water in ethanol, acetone, and methanol extraction solvents extracted high amounts of flavonoids and phenolics.[28] This showed how different factors could be a factor for the plant's phytochemical and biological activity.

The nature of the phytochemical constituents found in the extracts had exhibited akapulko's possible antifungal activity. Compounds such as the flavones 2,5,7,4'-tetrahydroxy isoflavone and 3,5,7,4'-tetrahydroxy flavone effectively inhibited *T. schoenleinii, T. longiforus, Pseudallescheria boydii, C. albicans,* and *A.* niger.[30] In addition, anthraquinones such as aloe-emodin and emodin inhibited the growth of *T. rubrum, T. mentagrophytes,* and *M. gypseum*[18,31,32] while cannabinoid alkaloid (4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol) from ethanolic seed extract inhibited *C. albicans* and *A. niger* growth.[33] Table 1 summarizes the phytochemicals detected from the different parts of akapulko using different types of solvents.

There has been very few published works on the mechanism of action of akapulko. Determining the molecular mechanism of action especially for crude herbal extracts is difficult because there are thousands of compounds present that can either act synergistically or antagonistically with each other. In three *Cassia* species (*C. alata, C. fistula,* and *C. tora*), the extracts inhibited hyphal growth in *T. rubrum, M. gypseum,* and *P. marneffei,* as well as the conidial growth in *M. gypseum*. The observed shrinking of the macroconidia and hyphae of extract-treated *M. gypseum* suggested cell leakage and change in membrane stability.[48] Abnormality in the cell wall was also observed in methicillin-resistant *S. aureus* cells[34] upon treatment using the extract. In other plants, berberine (an alkaloid) destabilizes the cell wall and the synthesis of ergosterol and causes mitochondrial dysfunction in *C.* albicans.[49] Alkaloids can also inhibit alpha-amylase in *A. niger* preventing the conversion of starch to usable forms of energy, impeding fungal growth.[50] At a molecular level, *in silico* molecular docking, and *in vitro* HPLC-UV analyses showed that the compound astragalin (kaempferol-3-*O*-β-D-glucopyranosi de) binds to the DNA at the G-C base pairs.[51] Moreover, in *S. cerevisiae* mutants lacking enzymes for antioxidant synthesis, DNA repair enzymes (RAD3, RADS2, and RAD6) or membrane constituents showed high sensitivity towards the akapulko extract.[51]

Table 1. Summary of the phytochemicals extracted from different plant parts of *Senna alata* Linn. using various solvent systems

Chemical Compound	Plant Part	Extraction Solvent
Alkaloid	Fresh and dried leaves	96% ethanol[34]
	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf, stem, root	Methanol, aqueous[36]
	Leaf	Ethanol[37]
	Leaf	Methanol, chloroform, petroleum ether, water[38]
Anthracenosides	Leaf	Methanol[39]
Anthraquinone	Leaf	Methanol, chloroform, petroleum ether, water[38]
β-sitosterol	Leaf	Methanol[40]
Cardioglycoside	Leaf, stem, root	Acetone[36]
Coumarin	Leaf	50% ethanol, 50% methanol, aqueous[35]
Flavonoids	Fresh and dried leaves	96% ethanol[34]
	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf, stem, root	Methanol, acetone, aqueous[36]
	Leaf	Ethanol[37]
	Leaf	Methanol, chloroform, petroleum ether, water[38]
	Leaf	Water, benzene, chloroform, methanol, petroleum ether[41]
Gallic tannins	Leaf	Methanol[39]
Glycosides	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf, stem, root	Methanol, acetone, aqueous[36]
	Leaf	Methanol, chloroform, petroleum ether, water[38]
Gum	Leaf, stem, root	Aqueous[36]
Lipids		
Fat oil	Leaf, stem, root	Acetone, chloroform[36]
Linoleic acid	Leaf	Methanol[40]
Linolenic acid	Leaf	Methanol[40]
Monosaturated fatty acids	Leaf	Methanol[40]
Myriatic acid	Leaf	Methanol[40]
Oleic acid	Leaf	Methanol[40]
Palmitic	Leaf	Methanol[40]
Polyunsaturated fatty acids	Leaf	Methanol[40]
Saturated fatty acids	Leaf	Methanol[40]
Stearic acid	Leaf	Methanol[40]
Mucilage	Leaf, stem, root	Aqueous[36]
Phenols	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf	Ethanol[37]
	Leaf	Water, benzene, chloroform, methanol, petroleum ether[41]
Phytosterol	Leaf, stem, root	Chloroform, acetone, methanol[36]
Proanthocyanidin	Leaf	Benzyl mercaptan[42]
Quinone	Dried and fresh leaves	96% ethanol[34]
	Leaf, stem, root	Chloroform, acetone, methanol, water[36]
Resin	Leaf, stem, root	Chloroform, Acetone[36]
Saponins	Fresh and dried leaves	96% ethanol[34]
	Leaf	50% ethanol, aqueous[35]
	Leaf, stem, root	Aqueous[36]
	Leaf	Ethanol[37]
	Leaf	Methanol, chloroform, petroleum ether, water[38]
	Leaf	Methanol[39]
Steroids	Fresh and dried leaves	96% ethanol[34]
	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf	Ethanol[37]

Tannins	Fresh and dried leaves	96% ethanol[34]
	Leaf	50% ethanol, 50% methanol, aqueous[35]
	Leaf, stem, root	Methanol, acetone, aqueous[36]
	Leaf	Ethanol[37]
	Leaf	Methanol, chloroform, petroleum ether, water[38]
Terpenoid	Leaf	50% ethanol, 50% methanol, aqueous[36]
	Leaf, stem, root	Methanol, acetone, aqueous[37]
Volatile oil	Leaf	Methanol, chloroform, petroleum ether, water[38]
Specific Compounds		
2,5,7,4'-tetrahydroxyisoflavone	Leaf	80% ethanol[30]
3,5,7,4'-tetrahydroxyflavone	Leaf	80% ethanol[30]
Aloe-emodin	Leaf	80% methanol[43]
Cassiaindoline	Leaf[44]	
Danthron	Leaf	Water[45]
Dihydroxycinnamic acid	Leaf	10:1 acetone:ethanol[46]
Kaempferol	Leaf	80% methanol[43]
	Leaf	Water[44]
	Leaf, stem, bean	Methanol[47]
Kaempferol-3-*O*-β-glucopyranoside	Leaf	80% methanol[43]
Kaempferol-3-*O*-gentioside	Leaf	80% methanol[43]
Kaempferol-*O*-glucoside	Leaf	Water[45]
Luteolin	Leaf, stem, bean	Methanol[47]
Quercetin-*O*-glucoside	Leaf	Water[45]
Rhein	Leaf	Water[45]

In vitro studies confirming the antifungal properties of *Senna alata*

In vitro studies showed that akapulko had anti-bacterial,[36,44,52,53,54] anti-inflammatory,[55,56] anti-oxidant,[37,40,57,58] hepatoprotective,[59] cardioprotective,[60] anti-tumor[61,62,63] and anti-malarial[64] properties. The most extensively studied property of akapulko was its antifungal ability. In the Philippines, ointments and soaps were formulated to target skin fungal diseases.[14] Table 2 summarizes the majority of the *in vitro* antifungal testing done on akapulko against a wide range of fungal species. As shown in the table, the different plant parts of akapulko extracted using different types of solvents showed varying antifungal properties against common fungal pathogens. Specifically, there is a wide range in the activities observed using agar well diffusion and broth dilution method. Different studies also had varying results, which could probably be due to the differences in the environmental and laboratory conditions during the *in vitro* testing, type of soil used where the plant was cultivated, weather condition during the time of sampling, and even the species of fungi being tested. It can also be observed that isolating a specific compound and using it to kill the fungal species showed lower activity compared with the crude extract. This was true for the activity of leaf anthraquinone aglycone against *E. flocossum* (29-fold lower), *M. gypseum* (31-fold lower), *T. metangrophytes* (58-fold lower), and *T. rubrum* (99-fold lower). These results entail the presence of compounds present in the herbal crude extract that act synergistically with each other to achieve maximum pharmacologic effect. This is also suggestive of the benefit of using herbal crude extracts for medication over synthetically-produced, individual compounds.

In general, ethanol extracts showed better activity compared to methanol, hexane, and aqueous extracts among all fungal species. Good antifungal activity was generally seen for *Aspergillus spp, Candida albicans, Cryptococcus neoformans, Epidermophyton flocossum, Microsporum spp., Trichophyton spp,* and *Penicillium marneffei* with MICs ranging from 3.5 mg/mL to 125 mg/mL. Such concentrations were achievable for topical formulations, thus akapulko is suited to treat cutaneous fungal infections.

Evidence of Efficacy and Safety in Humans

In the Philippines, several clinical trials and formulations were done in hospitals and laboratories to determine the efficacy of akapulko in human fungal infections. A systematic review was recently performed involving seven randomized controlled trials (RCT) wherein patients were treated with formulations of akapulko then compared with other antifungal agents.[75] There was a comparable mycologic cure between akapulko and 25% sodium thiosulfate in four RCTs. The two RCTs showed an efficacy similar to terbinafine and ketoconazole. Adverse effects were mild in all treatment groups. This systematic review is also featured in this issue of Acta Medica Philippina and thus the clinical trials will not be discussed in detail in this review.

Table 2. Summary of the results of *in vitro* antifungal studies for *Senna alata* Linn.

Fungi	Antifungal assay, outcome measured	Plant part – Extraction Solvent Used	Result
Aspergillus flavus	AWD, ZOI	Leaf – Ethanol[65]	22.1±0.1mm
	AWD, ZOI	Leaf – Aqueous[65]	20.1±0.1 mm
	AWD, ZOI	Leaf – Methanol[66]	10-20mm
	AWD, ZOI	Leaf – Aqueous[66]	10-20mm
	BM, MIC	Flower – Aqueous [67]	10mg/mL
	Extrapolated from AWD	Root – Methanol[68]	50 mg/mL
	Extrapolated from AWD	Leaf – Methanol[68]	50 mg/mL
Aspergillus niger	AWD, ZOI	Leaf – Ethanol[65]	25.2±0.3 mm
	AWD, ZOI	Leaf – Aqueous[65]	27.2±0.2 mm
	BM, MIC	Root – Methanol[69]	50 mg/mL
	BM, MIC	Leaf – Methanol[69]	50 mg/mL
	Extrapolated from AWD	Leaf – Ethanol[68]	3.5 mg/mL
	Extrapolated from AWD	Leaf – Aqueous[68]	32.4 mg/mL
Aspergillus parasiticus	BM, MIC	Flower – Aqueous[67]	15 mg/mL
Candida albicans	AWD, ZOI	Leaf – Ethanol[65]	18.2±0.2 mm
	AWD, ZOI	Leaf – Aqueous[65]	14.1±0.1 mm
	DD, ZOI at20mg/mL	Leaf – Methanol[66]	10-20mm
	DD, ZOI at20mg/mL	Leaf – Aqueous[66]	10-20mm
	Extrapolated from ZOI	Leaf – Ethanol[68]	5.6 mg/mL
	Extrapolated from ZOI	Leaf – Aqueous[68]	26.9 mg/mL
	BM, MIC	Leaf – Methanol[69]	35 mg/mL
	BM, MIC	Root – Methanol[69]	25mg/ml
	AWD, ZOI at 50 mg/mL	Leaf – Ethyl acetate[70]	12 mm
	AWD, ZOI at 50 mg/mL	Leaf – Hexane[70]	15 mm
	BM, MIC	Flower – Aqueous[67]	15 mg/mL
Cryptoccocus neoformans	BM, MIC	Root – Methanol[69]	6 mg/mL
	BM, MIC	Leaf – Methanol[69]	13 mg/mL
Epidermophyton floccosum	BM, MIC	Stem bark – Ethanol[71]	2.5 mg/mL
	BM, MIC	Leaf – Ethanol[31]	3.75 mg/mL
	BM, MIC	Leaf – Antraquinone aglycone from glycosidic fraction[31]	0.13 mg/mL
Microsporum canslaslomyces	BM, MIC	Stem bark – Ethanol[71]	2.5 mg/mL
Microsporum canis	BM, MIC	Leaf – Ethanol[72]	62.5 mg/mL
	Extrapolated from ZOI	Leaf – Ethanol[68]	12.6 mg/mL
	Extrapolated from ZOI	Leaf – Aqueous[68]	30.30 mg/mL
Microsporum gypseum	BM, MIC	Leaf – Ethanol[72]	62.5 mg/mL
	BM, MIC	Leaf – Ethanol[31]	10.42 mg/mL
	BM, MIC	Leaf –Anthraquinone aglycone from glycosidic fraction[31]	0.34 mg/mL
	100% hyphal growth inhibition, IC_{50}	Leaf – Methanol[48]	10mg/mL, 0.8 mg/mL
Microsporum audouinii	AWD, ZOI	Leaf – Hexane[22]	25mm
	BM, MIC	Leaf –Ethyl Acetate[22]	22 mm
	BM, MIC	Flower – Aqueous[67]	15 mg/mL
	BM, MIC	Leaf – Methanol[73,74]	25 mg/mL
Trichophyton verrucosum,	BM, MIC	Stem bark – Ethanol[71]	2.5 mg/mL
Trichophyton megnini	BM, MIC	Leaf – Methanol[75]	50 mg/mL

Trichophyton mentagrophytes	BM, MIC	Stem bark – Ethanol[71]	2.5 mg/mL
	BM, MIC	Leaf – Ethanol[31,72]	125 mg/mL
	BM, MIC	Leaf – Ethanol[68]	9.8mg/mL
	BM, MIC	Leaf –Aqueous[68]	27.8mg/mL
	Extrapolated from ZOI BM, MIC	Leaf – Ethanol[31]	19.64 mg/mL
	Extrapolated from ZOI BM, MIC	Leaf –Anthraquinone aglycone from glycosidic fraction[31]	0.34mg/mL
	BM, MIC	Leaf – Methanol[74]	14 mm
	AWD, ZOI at 50 mg/mL	Leaf – Hexane[70]	16 mm
	AWD, ZOI at 50 mg/mL	Leaf – Ethyl acetate[70]	22 mm
	AWD, ZOI at 50 mg/mL	Leaf –Chloroform[70]	23mm
	AWD, ZOI	Leaf – Hexane[22]	22mm
Trichophyton rubrum	BM, MIC	Leaf – Ethanol[31]	18.75 mg/mL
	BM, MIC	Leaf –Anthraquinone aglycone from glycosidic fraction[31]	0.19 mg/mL
	BM, MIC	Leaf – Methanol[73]	50 mg/mL
	DD, ZOI 20 mg/mL	Leaf – Methanol[67]	10-20mm
	100% hyphal growth inhibition, IC_{50}	Leaf – Methanol[48]	10 mg/mL, 0.5 mg/mL
Trichophyton tonsurans	BM, MIC	Leaf – Methanol[73]	50 mg/mL
Penicillium marneffei	100% hyphal growth inhibition, IC_{50}	Leaf – Methanol[48]	100mg/mL, 6.6 mg/mL

AWD – agar well diffusion
ZOI – zone of inhibition
DD – disc diffusion
BM – broth microdilution
MIC – minimum inhibitory concentration

CONCLUSION AND RECOMMENDATIONS

Senna alata (akapulko) has been well-studied for its antifungal activity. Because of this, it is one of the ten herbal medicines endorsed by the Philippine Department of Health. Several *in vitro* and clinical studies confirmed its efficacy, thus, providing an evidence-based claim that can support and strengthen the use of this plant for the treatment of skin fungal indications. Still, additional studies on the formulation of the extract must be done, especially for these cases where fungal strains constantly change, phenotypically and genetically. Current formulations may not be effective in the coming years. Also, the cellular and molecular mechanisms of action of the plant extract must be established. Knowing the bioactive compounds can be of use to biotechnologists and breeders to produce plant varieties that can produce more of these compounds or can grow more of the plant parts collected for extraction that can contribute largely in conserving our resources. Lastly, the results of the molecular mechanism of action studies may give essential results in the formulation of personalized medications and treatment.

Statement of Authorship

All authors participated in data collection and analysis, and approved the final version submitted.

Author Disclosure

All authors declared no conflict of interest. The NIRPROMP-IHM has a utility model on an akapulco antifungal product.

Funding Source

This paper was funded by the authors.

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