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RESEARCH ARTICLE

Antifungal properties of soleshine - An *in vitro* study

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ABSTRACT

Background: Soleshine is a polyherbal formulation meant to be used to heal cracks on heels, protecting sole from chaffs, marketed by Rumi Herbal Pvt Ltd., Chennai. The active ingriedients are *Azadirachta indica, Lawsonia alba, Shorea robusta, Ricinus communis,* and *Sesamum indicum,* which were well known for their antimicrobial activity. **Aim and Objectives:** The aim of this study is to evaluate the antifungal activity of the ingredients and to evaluate the synergistic activity present in the preparation. **Materials and Methods:** The plant materials were procured, dried, powdered, and sequentially extracted. The organisms were collected and evaluated the antifungal activity with disc diffusion method for all individual extracts and in combination of different extracts. Sabouraud dextrose agar, culture medium was used for the growth of fungal organism. The minimum inhibitory activity was observed with resazurin method. Clotrimazole, an antifungal drug, belongs to azole group was taken as a standard negative control. **Results:** The zone of inhibition was determined by disc diffusion method where it shows the inhibition of growth of fungal organism. The same experiment was repeated totally for 6 times and analyzed with SPSS software in May 2017, found that significant ($P < 0.05$). The minimum amount of extract required to inhibit the growth was determined by resazurin dye method where the color change (oxidation-reduction reaction) implies the viability of fungi. **Conclusion:** The formulation has an antifungal activity in all the extracts and maximum activity was with aqueous extract, and also, it shows synergistic activity in combination which quotes the additive effect.

KEY WORDS: Soleshine; Dermatophytes; Antifungal Activity; Minimum Inhibitory Concentration; Synergism

INTRODUCTION

Herbal and naturally obtaining products as a traditional medicine have been used for centuries ago around the world. Research scholars and medical scientists have inclined in the arena of herbal medicines as they discovered the true real health benefits. "Let food be your medicine and let medicine be your food" was advised by the father of medicine, Hippocrates, over a millennium ago.[1] The plants and its products used in the treatment of skin diseases including mycotic infections are an age-old practice in all parts of the world, especially in India.[2]

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Soleshine is a polyherbal preparation formulated by Rumi Herbals used traditionally for fungal infections like *Tinea pedis*. This preparation was composed of five plant extracts, among which three were known for antimicrobial activity. The five plants or plant products present as ingredients of

polyherbal preparation were *Azadirachta indica*, *Lawsonia alba*, *Shorea robusta*, *Ricinus communis*, and *Sesamum indicum*.

A. indica was synonymously known as neem plant in English, used to control infections such as leprosy, intestinal helminthiasis, chronic sores infected by syphilis, and respiratory disorders.[3] Lotion isolated from neem leaf, when applied externally, can cure many dermatological infections within 3–4 days.[4]

Lawsonia inermis, commonly called as henna, widely distributed in middle East, Sahel, and Central Africa.[5] The powdered leaves of henna are used for staining hair, nails, and beard.[6] The leaves of *L. inermis* are used in the treatment of poliomyelitis and measles among the Yoruba tribe of South Western Nigeria.[7] The seeds of *Lawsonia* have been reported to present deodorant action and are used in many cases of gynecological disorders such as menorrhagia, vaginal discharge, and leukorrhea.[8]

S. robusta was synonymously known as Sal tree belongs to the family *Dipterocarpaceae*,[9] the resin shows analgesic[10] and antipyretic[11] activity in 70% ethanol extract, whereas methanol and aqueous leaf extract shows anti-inflammatory activity.[12-14] The methanol extract of the dried leaves was used as an antinociceptive activity, aqueous extract of floral parts was used as antibacterial,[11] and anti-obesity effect was observed in hydroalcoholic extract of the leaves.[15] The aqueous, methanol, petroleum, and benzene extract of oleoresin inhibits the growth of microorganisms.[16] Ethanolic extract (10 and 30% w/w) applied in excised and incised wounds produces a dose-dependent acceleration of wound healing.[17]

Castor oil also known as *R. communis*, used in the treatment of warts, cold tumors, indurations of the mammary glands, corns and moles,[18-21] the anti-inflammatory, and free radical scavenging activity was also observed.[22]

Sesame oil is botanically known as *S. indicum* used as analgesic, antipyretic, and anti-inflammatory activities in animal models.[23] Sesamin, a secondary metabolite, decreases cholesterol levels as well as increases high-density lipoprotein levels.[24] Sesame is a naturally available antibacterial agent for common pathogens of skin and common skin fungal infections like athlete's foot.[25] Sesamin also used as an antihypertensive agent, evidenced in experimental animal models.[26] Sesame oil has an antioxidant property that helps to prevent oxidative damage and enhances the healing process of damaged tissues.[27,28]

The plants used in this study were well known for antimicrobial activity, and this study shows the antifungal activity of individual plants and synergism. The combination of more than one plant extract may show more effective than individual, with minimum dose. This study was aimed to evaluate the antifungal activity and the synergistic activity of the *A. indica, L. alba,* and *S. robusta* present in soleshine, a polyherbal preparation on dermatophytes and *Candida* with individual drugs and in combination.

MATERIALS AND METHODS

Collection of Plant Material

The matured leaves of *A. indica* and *L. alba* were identified, collected from the premises of Tagore Medical College and Hospital campus, and authenticated by a botanist, resin of *S. robusta,* castor oil, and sesame oil was purchased from Rumi Herbals, Chennai, with a certificate of analysis.

Preparation of Extracts

The procured leaves of *A. indica* and *L. alba* were washed with distilled water and dried in shadow area for 2 weeks and smashed by mechanical grinder then passed through a 20 mesh sieve. The powdered leaf was sequentially extracted with hexane, benzene, ethyl acetate, and methanol, using Soxhlet apparatus and aqueous with cold maceration. The extraction was carried out for 1 day in normal room temperature with mild shaking. The extracts were filtered and concentrated at 35°C, and the weight of each residue was recorded and stored in 4°C.

Procurement of Microorganisms

The organisms were procured from the microbial type culture collection and Gene bank, Chandigarh. The antifungal activity was screened using dermatophytes and the *Candida albicans* (MTCC code: 3017) species. The three dermatophytes used for screening were *Trichophyton rubrum* (MTCC code: 7859), *Microsporum gypseum* (MTCC code: 4524), and *Epidermophyton floccosum* (MTCC code: 7880).

Determination of Antifungal Activity

The disc diffusion method[29,30] is a method that is suited for the organisms that grow rapidly overnight. The impregnated disc imbibes moisture from the agar where the sample or fungicidal extract diffuses into the agar medium. A zone of inhibition of the sample against fungal growth around each disc was measured and the susceptibility was determined. Disc diffusion method[29,30] was the standard method for carrying out the antimicrobial analysis using 100 µl of a suspension containing 106 spores/ml of fungal organisms which spread on sabouraud dextrose agar (SDA).

SDA was prepared, autoclaved at 121°C for 15 min at 15 lbs, and poured into sterile Petri-plates with a uniform thickness of approximately 5–6 mm, and the agar was allowed to set at ambient temperature.

Each Petri dish was inoculated with any one of the fungal cultures suitably diluted to contain more than 106 cells per ml by spreading 0.1 ml suspension of the organism with sterile cotton swab. On each plate, a 6 mm diameter of a non-contaminated sterile disc was placed. With the help of micropipette, the 10 µl (1 mg/ml) of the extract was taken and placed on the disc. Then, it was subjected to check the zone of inhibition. Clotrimazole was used as a standard.

Minimum Inhibitory Concentration (MIC)

The MIC was detected by the resazurin microtiter method.[31] 100 µl of SDA broth was taken and placed in each well of 96 well microtiter plate, with the capacity of 200 µl in each well. In well-sterilized conditions, the freshly grown microorganism was placed in all 96 well microtiter plate and labeled properly. Serial dilutions of all the extracts were made and placed in an appropriate microtiter plate. Simultaneously, 5 µl resazurin indicator (prepared by dissolving 270 mg tablet in 40 µl of sterile distilled water) was also added in all 96 wells. Shake it very gently to ensure that all wells are homogeneously mixed. Finally, the microtiter plate was kept in the dark place to avoid interference of light. It was shaken once in 24 h. After 48 h, the color change from violet (oxidized state) to colorless or pink (reduced state) was observed. Clotrimazole mixed in 5% dimethyl sulfoxide (DMSO) used as a standard.

RESULTS

The various extracts of the plants were tested for the growth of the fungi using different concentrations, where DMSO was used as a positive control. The clotrimazole, a standard drug for fungal infections, was used in this study as a negative control.

Determination of Antifungal Activity by Disc Diffusion Method

The zone of inhibition was taken as a parameter to evaluate the fungicidal activity of the extract. The same experiment was repeated totally for 6 times, then the results were analyzed using one-way ANOVA (analysis of variance) and Wilk's Lambda *P* value using software, IBM SPSS Version: 20, and they were showing a highly significant ($P < 0.05$).

The values in Table 1 represent the mean of various extracts for *A. indica*. The results for various extracts of *L. alba* and *S. robusta* were tabulated in Tables 2 and 3, respectively. The synergistic action of the three plants in combination was tabulated in Table 4.

Determination of MIC

The MIC required for sequential extracts of *A. indica, L. alba,* and *S. robusta* [Figure 1] and for soleshine [Figure 2] to inhibit the growth of the fungal organisms was performed by resazurin method. The chemical name of resazurin is 7-hydroxy-3H-phenoxazin-3-one 10-oxide which is a dye. Resazurin was irreversibly reduced from violet to pink color which is a fluorescent compound, resorufin[32] which acts as an indicator to check the cell viability. The color change observed at certain concentration shows the minimum concentration required to inhibit the growth of the fungi.

DISCUSSION

Soleshine is a polyherbal preparation composed of leaf extract of *A. indica*, leaf extract of *L. alba*, resin of *S. robusta*, castor oil, and sesame oil. The neem, henna, and sal resin were well known for the antimicrobial activity, while the castor oil

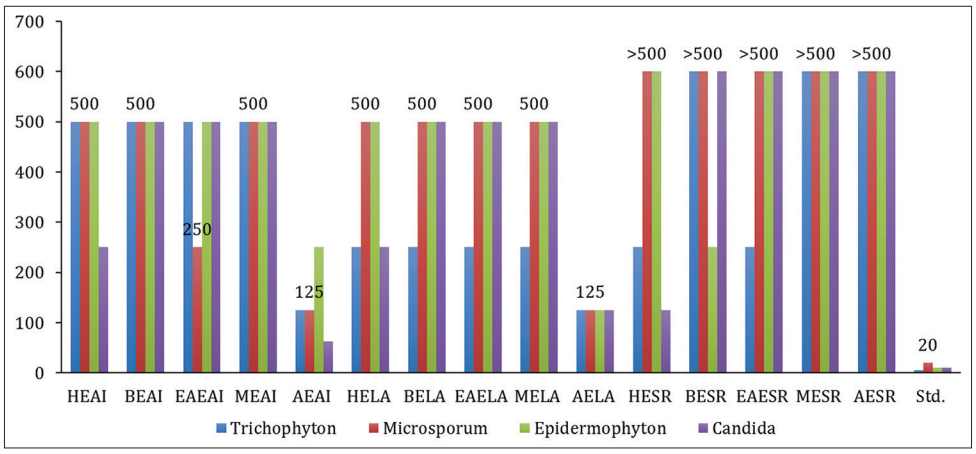

Figure 1: The minimum inhibitory concentration of sequential extracts of *Azadirachta indica, Lawsonia alba,* and *Shorea robusta.* HEAI: Hexane extract of *A. indica*, BEAI: Benzene extract of *A. indica*, EAEAI: Ethylacetate extract of *A. indica*, MEAI: Methanol extract of *A. indica*, AEAI: Aqueous extract of *A. indica,* HELA: Hexane extract of *L. alba*, BELA: Benzene extract of *L. alba,* EAELA: Ethylacetate extract of *L. alba,* MELA: Methanol extract of *L. alba,* AELA: Aqueous extract of *L. alba,* HESR: Hexane extract of *S. robusta*, BESR: Benzene extract of *S. robusta*, EAESR: Ethylacetate extract of *S. robusta,* MESR: Methanol extract of *S. robusta,* AESR: Aqueous extract of *S. robusta,* Std: Standard

Table 1: The average of various extracts of ***A. indica***

Extract	Organism	Zone of inhibition (mm)					
		50 ug/ml±SD	100 ug/ml±SD	250 ug/ml±SD	500 ug/ml±SD	Vehicle control ug/ml	Standard (clotrimazole) ug/ml
HEAI	*Trichophyton*	6.93±0.018	7.28±0.053	10.28±0.043	13.14±0.016	NI	19.30
	Microsporum	6.23±0.049	6.62±0.017	6.33±0.017	6.88±0.013	NI	20.15
	Epidermophyton	6.90±0.040	7.48±0.012	8.29±0.024	8.44±0.017	NI	21.58
	Candida	NI	NI	NI	NI	NI	19.99
BEAI	*Trichophyton*	6.46±0.016	7.64±0.020	7.48±0.012	10.68±0.008	NI	19.30
	Microsporum	6.19±0.016	7.37±0.020	7.70±0.010	10.54±0.023	NI	20.15
	Epidermophyton	6.35±0.016	6.54±0.021	7.08±0.069	6.66±0.019	NI	21.58
	Candida	NI	6.53±0.030	6.74±0.016	7.77±0.019	NI	19.99
EAEAI	*Trichophyton*	6.06±0.019	6.55±0.020	6.87±0.010	8.32±0.019	NI	19.30
	Microsporum	6.26±0.024	7.53±0.048	7.94±0.017	8.42±0.014	NI	20.15
	Epidermophyton	6.55±0.051	7.86±0.045	8.13±0.032	7.42±0.037	NI	21.58
	Candida	NI	NI	7.24±0.017	9.65±0.025	NI	19.99
MEAI	*Trichophyton*	6.52±0.019	7.46±0.028	8.13±0.012	8.43±0.018	NI	19.30
	Microsporum	6.53±0.023	8.93±0.025	9.97±0.018	10.30±0.061	NI	20.15
	Epidermophyton	6.06±0.021	6.84±0.022	7.23±0.021	7.42±0.024	NI	21.58
	Candida	NI	6.82±0.014	7.41±0.011	8.16±0.022	NI	19.99
AEAI	*Trichophyton*	7.13±0.027	7.80±0.014	8.03±0.038	8.14±0.017	NI	19.30
	Microsporum	7.13±0.017	8.74±0.023	9.34±0.017	10.69±0.021	NI	20.15
	Epidermophyton	6.33±0.056	7.57±0.027	7.83±0.097	7.95±0.023	NI	21.58
	Candida	7.07±0.016	7.42±0.014	11.57±0.026	12.23±0.023	NI	19.99

HEAI: Hexane extract of *Azadirachta indica*, BEAI: Benzene extract of *Azadirachta indica*, EAEAI: Ethyl acetate extract of *Azadirachta indica*, MEAI: Methanol extract of *Azadirachta indica,* AEAI: Aqueous extract of *Azadirachta indica*, NI: No inhibition. *A. indica: Azadirachta indica,* SD: Standard deviation

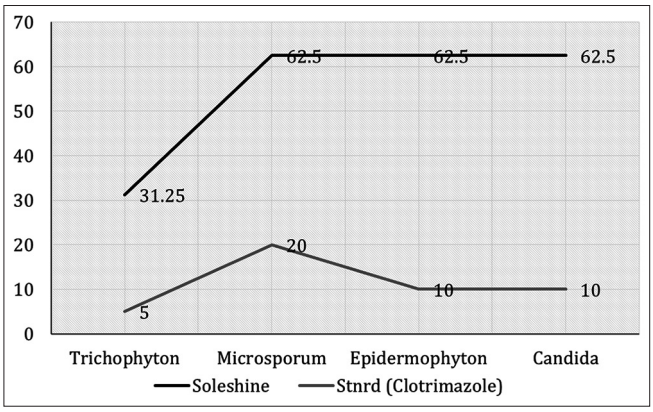

Figure 2: The minimum inhibitory concentration for a polyherbal preparation

and sesame oil were used as vehicles for the preparation of formulation.

In the present study, the aqueous extract of *A. indica*, and *L. alba* exhibited a maximum zone of inhibition against all dermatophytes and *Candida* in a dose-dependent acceleration when compared with the other extracts [Tables 1-3]. The combination of aqueous extract of neem, henna, and sal resin exhibits synergism [Table 4].

The values in Table 1 represent the mean of various extracts for *A. indica*. The hexane extract of neem at 500 ug/ml shows 13.14 mm zone of inhibition against Trichophyton, the benzene extract at 500 ug shows 10.68 mm against Trichophyton, ethyl acetate extract at 500 ug shows 9.65 mm against *Candida*, the methanol extract at 500 ug shows 10.30 mm against Microsporum, and the aqueous extract at 500 ugs shows 12.23 mm against *Candida*. However, in all four organisms, aqueous extract of neem has a zone of inhibition, and it was subjected to consider for synergistic activity. The results for various extracts of *L. alba* were tabulated in Table 2. The hexane extract of henna at 500 ug/ml shows 10.57 mm zone of inhibition against Trichophyton, the benzene extract at 500 ug shows 10.66 mm against Microsporum, ethyl acetate extract at 500 ug shows 10.30 mm against Microsporum, the methanol extract at 500 ug shows 9.11 mm against Microsporum, and the aqueous extract at 500 ugs shows 11.25 mm against *Candida*. However, in all four organisms, aqueous extract of henna has a zone of inhibition, and it was considered to know the synergistic activity. The results for various extracts of *S. robusta* were tabulated in Table 3. No inhibition (NI) was observed against dermatophytes with all extracts. However, against *Candida,* the zone of

Table 2: The average of various extracts of ***L. alba***

Extract	Organism	Zone of inhibition (mm)					
		50 ug/ml±SD	100 ug/ml±SD	250 ug/ml±SD	500 ug/ml±SD	Vehicle control ug/ml	Standard ug/ml
HELA	*Trichophyton*	6.06±0.014	6.92±0.024	7.70±0.014	10.57±0.035	NI	19.30
	Microsporum	6.24±0.029	6.24±0.024	6.71±0.011	6.83±0.021	NI	20.15
	Epidermophyton	6.11±0.016	6.25±0.014	6.95±0.021	8.70±0.012	NI	21.58
	Candida	NI	6.06±0.014	6.15±0.013	6.35±0.020	NI	19.99
BELA	*Trichophyton*	6.28±0.012	6.32±0.012	6.39±0.032	6.57±0.011	NI	19.30
	Microsporum	6.18±0.021	6.35±0.025	8.69±0.041	10.66±0.026	NI	20.15
	Epidermophyton	6.31±0.012	6.52±0.013	6.93±0.008	6.96±0.014	NI	21.58
	Candida	NI	6.32±0.016	6.61±0.059	7.60±0.020	NI	19.99
EAELA	*Trichophyton*	6.32±0.020	6.47±0.013	6.52±0.011	6.72±0.011	NI	19.30
	Microsporum	6.12±0.014	6.67±0.012	8.27±0.012	10.30±0.012	NI	20.15
	Epidermophyton	6.24±0.014	6.43±0.014	6.60±0.153	6.86±0.020	NI	21.58
	Candida	6.18±0.014	6.37±0.012	6.85±0.017	7.13±0.013	NI	19.99
MELA	*Trichophyton*	6.22±0.013	6.33±0.017	6.42±0.014	6.83±0.010	NI	19.30
	Microsporum	6.22±0.016	6.55±0.012	7.75±0.040	9.11±1.221	NI	20.15
	Epidermophyton	6.26±0.016	6.31±0.012	6.61±0.016	6.89±0.118	NI	21.58
	Candida	NI	6.46±0.012	7.02±0.012	7.42±0.013	NI	19.99
AELA	*Trichophyton*	6.06±0.017	7.60±0.012	8.13±0.014	10.04±0.031	NI	19.30
	Microsporum	6.44±0.020	7.16±0.014	8.43±0.011	10.38±0.218	NI	20.15
	Epidermophyton	6.57±0.015	6.85±0.020	8.32±0.013	10.47±0.012	NI	21.58
	Candida	8.67±0.012	9.41±0.011	10.81±0.014	11.25±0.017	NI	19.99

HELA: Hexane extract of *Lawsonia alba*, BELA: Benzene extract of *Lawsonia alba,* EAELA: Ethyl acetate extract of *Lawsonia alba*, MELA: Methanol extract of *Lawsonia alba,* AELA: Aqueous extract of *Lawsonia alba*, NI: No Inhibition. *L. alba: Lawsonia alba*, SD: Standard deviation

inhibition was 10.75 mm at 500 ug/ml in hexane extract, 10.04 mm for benzene extract in 500 ug, 7.94 mm at 250 ug for ethyl acetate, 8.61 mm for methanol extract at 500 ug, and 6.54 mm for aqueous extract at 500 ug. The aqueous extract of sal resin was considered to know the synergistic activity being it was known for antimicrobial activity by previous workers, even it does not show activity in dermatophytes in this study.

The synergistic action of the three plants in combination was tabulated in Table 4. The maximum zone of inhibition was observed at 15.85 mm, 13.80 mm, and 16.25 mm against *Trichophyton*, *Microsporum*, and *Epidermophyton,* respectively, at 500 ug/ml but for *Candida* 13.67 mm was at 100 ug.

The MIC of the sequential extracts for all extracts was shown in Figure 1 and for soleshine was shown in Figure 2.

In *A. indica,* the aqueous extract shows the minimum quantity required to inhibit the growth as 125 ug/ml against *Trichophyton* and *Microsporum*, 250 ug for *Epidermophyton,* and 62.5 ug for *Candida*. In *L. alba,* the aqueous extract was exhibiting minimum quantity required to inhibit the growth as 125 ug/ml in *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Candida*. In *S. robusta*, the minimum quantity required was 250 ug/ml against *Trichophyton* and 125 ug against *Candida* for hexane extract [Figure 1].

The minimum quantity for formulation soleshine was 31.5 ug/ml against *Trichophyton* and 62.5 ug/ml for *Microsporum*, *Epidermophyton*, and *Candida* [Figure 2].

In a study by Mondali *et al*., neem leaf extract *in vitro* study exhibits the inhibition of radial growth of *Aspergillus* and *Rhizopus.*[33] In a study by Olufemi *et al.*, the neem seed oil shows the inhibition of growth of fungal organisms was more in *Curvularia* sp. than *Aspergillus* and *Fusarium* species. However, NI was seen against *Rhizopus stolonifera.*[34] In the present study, the aqueous extract of neem shows inhibition of growth of dermatophytes and *Candida*. According to Elmanama *et al*., methanolic extract of henna shows maximum activity than aqueous extraction,[35] but in the present study, the aqueous extract showed activity against all dermatophytes and *C. albicans* in a dose-dependent manner, probably it can be stated as the strains used in the study may have genetic differences which exhibit the alteration of results that can be proven by pharmacogenomic evaluation.

Table 3: The average of various extracts of ***S. robusta***

Extract	Organism	Zone of inhibition (mm)					
		50 ug/ml±SD	100 ug/ml±SD	250 ug/ml±SD	500 ug/ml±SD	Vehicle control ug/ml	Standard ug/ml
HESR	TRICHOPHYTON	NI	NI	NI	NI	NI	19.30
	MICROSPORUM	NI	NI	NI	NI	NI	20.15
	EPIDERMOPHYTON	NI	NI	NI	NI	NI	21.58
	CANDIDA	6.58±0.029	8.06±0.031	10.16±0.014	10.75±0.017	NI	19.99
BESR	TRICHOPHYTON	NI	NI	NI	NI	NI	19.30
	MICROSPORUM	NI	NI	NI	NI	NI	20.15
	EPIDERMOPHYTON	NI	NI	NI	NI	NI	21.58
	CANDIDA	NI	6.60±0.012	7.62±0.011	10.04±0.009	NI	19.99
EAESR	TRICHOPHYTON	NI	NI	NI	NI	NI	19.30
	MICROSPORUM	NI	NI	NI	NI	NI	20.15
	EPIDERMOPHYTON	NI	NI	NI	NI	NI	21.58
	CANDIDA	6.26±0.011	6.57±0.014	7.94±0.021	7.16±0.012	NI	19.99
MESR	TRICHOPHYTON	NI	NI	NI	NI	NI	19.30
	MICROSPORUM	NI	NI	NI	NI	NI	20.15
	EPIDERMOPHYTON	NI	NI	NI	NI	NI	21.58
	CANDIDA	NI	6.77±0.011	7.30±0.012	8.61±0.021	NI	19.99
AESR	TRICHOPHYTON	NI	NI	NI	NI	NI	19.30
	MICROSPORUM	NI	NI	NI	NI	NI	20.15
	EPIDERMOPHYTON	NI	NI	NI	NI	NI	21.58
	CANDIDA	6.04±0.011	6.25±0.013	6.30±0.018	6.54±0.022	NI	19.99

HESR: Hexane extract of *Shorea robusta*, BESR: Benzene extract of *Shorea robusta,* EAESR: Ethyl acetate extract of *Shorea robusta,* MESR: Methanol extract of *Shorea robusta,* AESR: Aqueous extract of *Shorea robusta*, NI: No inhibition. *S. robusta*: *Shorea robusta*, SD: Standard deviation

The addition of aqueous extract of *Hibiscus sabdariffa*[35] to ketoconazole and fluconazole drugs enhances the fungicidal activity. In a study by Murthy *et al*., the aqueous, methanol, petroleum, and benzene extracts of *S. robusta* show inhibition of the growth of various organisms[17] and the petroleum ether extract showed activity against *Escherichia coli*, *Aspergillus flavus,* and *C. albicans.* In the present study, NI was observed against dermatophytes, but activity was seen in *Candida*. According to Jombo and Enenebeaku,[36] it was observed that the castor oil has sensitivity in some organisms, but in this present study, it was observed NI against fungi. This castor oil was used as a base for the preparation of the polyherbal formulation. In a study by Saleem, it was found that the sesame oil has the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*, *Proteus vulgaris*, *Corynebacterium diphtheriae*, and *Streptomyces griseus*.[37] In this study, it was observed that the sesame oil has NI against dermatophytes and *Candida*.

The combination of aqueous extract of *A. indica*, aqueous extract of *L. alba*, and aqueous extract of *S. robusta* in equal concentration showed 15.85 mm, 13.80 mm, and 16.25 mm zone of inhibition at 500 ug/ml against *Trichophyton*, *Microsporum*, and *Epidermophyton* species, respectively, as 89%, 68%, and 75% with clotrimazole and 13.67 mm at 100 ug against *Candida* as 68% with clotrimazole which determines the synergistic activity [Table 4].

According to Radhika and Michael, the MIC for ethanolic and ethyl acetate extract of *A. indica* shows 125 ug/ml against *T. rubrum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, and *M. gypseum* while the hexane was 500 ug/ml.[38] In a study by Reddy *et al*., the MIC values of aqueous extract of *A. indica* leaves show 1000 ug/ml against *C. albicans* and 2000 ug/ml against *Aspergillus fumigatus*.[39] In the present study, the aqueous extract of *A. indic*a against *Trichophyto*n, *Microsporu*m, *Epidermophyto*n, and *Candid*a was 125 ug/ml, 125 ug/ml, 250 ug/ml, and 62.5 ug/ml for respectively [Figure 1].

According to Suleiman and Mohamed, the ethnolic extract of *L. inermis* shows that MIC was 7.5 mg/ml against *T. mentagrophytes*, 10 mg/ml for *Trichophyton verrucosum*, and 5 mg/ml for *Trichophyton violaceum*.[40] In a study by Sharma *et al*., the chloroform and methanol extract of *L. inermis* was >12.5 mg/ml against *T. mentagrophytes*,

Table 4: The average of aqueous extract of ***A. indica***±aqueous extract of ***L. alba***±aqueous extract of ***S. robusta***

Extract	Organism	Zone of inhibition (mm)							
		10 ug/ml±SD	**20 ug/ml±SD**	**50 ug/ml±SD**	**100 ug/ml±SD**	**250 ug/ml±SD**	**500 ug/ml±SD**	**Vehicle control µg/ml**	**Standard µg/ml**
AEAI+AELA+AESR	*Trichophyton*	7.17±0.058	8.08±0.041	9.54±0.034	10.57±0.049	13.95±0.046	15.85±0.032	NI	19.3
	Microsporum	7.44±0.029	8.85±0.028	10.82±0.036	12.79±0.240	12.05±0.036	13.80±0.040	NI	20.15
	Epidermophyton	6.81±0.40	7.34±0.048	9.94±0.035	10.65±0.038	15.05±0.027	16.25±0.044	NI	21.58
	Candida	6.91±0.026	8.09±0.064	11.02±0.023	13.67±0.046	13.14±0.025	13.62±0.027	NI	19.99

AEAI±AELA±AESR: Aqueous extract of *Azadirachta indica* ± Aqueous extract of *Lawsonia alba*±aqueous extract of *Shorea robusta*, NI: No inhibition. *A. indica: Azadirachta indica, L. alba: Lawsonia alba, S. robusta: Shorea robusta*, SD: Standard deviation

T. rubrum, *M. gypseum*, and *Microsporum fulvum* and the water extract was 6.25–12.5 mg/ml for *T. mentagrophytes* and *M. fulvum* and >12.5 mg/ml for *T. rubrum* and *M. gypseum*.[41] In this study, it was observed that the aqueous extract of *L. alba* was 125 ug/ml, 125 ug/ml, 125 ug/ml, and 125 ug/ml, respectively.

The quantity required for aqueous extract of *S. robusta* was >500 ug/ml, >500 ug/ml, >500 ug/ml, and >500 ug/ml against *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Candida*, and hexane extract was 250 ug/ml and 125 ug/ml against *Trichophyton* and *Candida,* respectively [Figure 1].

The minimum quantity required to inhibit against *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Candida* was observed as 31.5 ug/ml, 62.5 ug/ml, 62.5 ug/ml, and 62.5 ug/ml, respectively, for polyherbal preparation [Figure 2].

The limitation of the present study was a selection of microorganisms, only dermatophytes and *Candida* were selected. It needs to be evaluated in other pathogenic fungal organisms.

CONCLUSION

The findings in the present study ere shown as the individual extracts of polyherbal preparation were having an excellent antifungal activity, especially in dermatophytes and *Candida,* and in a combination of the different ingredients, it was observed as synergistic activity.

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