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PREPARATION AND EVALUATION OF ANTIFUNGAL PROPERTY OF A POLYHERBAL FORMULATION CONTAINING *ACHYRANTHES ASPERA* AND *PLECTRANTHUS AMBOENICUS*

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ABSTRACT: Research in complementary therapies and alternative medicines is increasing and formulating therapeutic medicines based on these findings has become the need of the hour. The present research aims to formulate and evaluate a polyherbal gel containing *Achyranthes aspera* and *Plectranthus amboenicus* extract. The formulation was designed by using alcoholic as well as aqueous extract of *Achyranthes aspera* and *Plectranthus amboenicus* leaves in varied concentrations. The extracts were subjected to various physicochemical evaluations. The gel was prepared by using a suitable gelling agent- Carbopol 934. The polyherbal formulation was evaluated for its antifungal activity and it was found that the formulation have elicited promising antifungal activity which was comparable with that of standard drug (fluconazole). However the activity was found to be due to the synergistic blend of herbal drugs. The polyherbal formulation exhibits a promising topical gel for fungal infections of the skin. Thus the present study demonstrates an immense scope for development of such polyherbal formulations and also explores the vast potential to further carry out research by exploiting synergistic effect in herbal extracts.

INTRODUCTION: Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality world wide¹. During the past several years there has been an increasing incidence of bacterial and fungal infections due to a growth in immuno-compromised population such as organ transplant, recipients, cancer and HIV/AIDS patient.

This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antimicrobial and antifungal drugs has been the reason for an extended search for newer drugs to treat opportunistic microbial or fungal infections².

Infections caused by pathogenic fungi are increasingly recognized as an emerging threat to public health. The incidence of opportunistic fungal infection in immuno-compromised host, especially in AIDS patients has increased drastically in recent years³. As compared with the synthetic products the herbal products are mild, biodegradable and have low toxicity profile⁴.

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Many substances separated from different extracts of the plants possessed antifungal activity⁵. However, reports on microbial inhibition by *Achyranthes aspera* and *Plectranthus amboenicus* are lacking.

Therefore, the present study was planned to evaluate antifungal property of Polyherbal formulation containing extracts of *Achyranthes aspera* and *Plectranthus amboenicus*.

MATERIALS AND METHODS:

Plant materials: The leaves of *Achyranthes aspera* and *Plectranthus amboenicus* were collected from local area of Lonavala and authenticated from Botanical Survey of India, Pune. The Voucher specimen number is (BSI/WC/Tech 273 and 274).

Pharmacognostic Study: The fresh leaves of *Achyranthes aspera* and *Plectranthus amboenicus* were screened for its morphological and microscopical characteristics⁶.

Physicochemical evaluation of leaves of each plant: The physicochemical evaluation was carried out using various parameters such as Ash value, extractive value, LOD etc.

Preparation of extracts: The fresh leaves of *Achyranthes aspera* and *Plectranthus amboenicus* were dried and powdered. The powdered leaves of each plant were subjected to successive solvent extraction by using Soxhlet assembly for obtaining chloroform and alcohol extract whereas the

maceration process was used for preparation of aqueous extract⁷.

Physicochemical evaluation of extracts: The alcohol and aqueous extracts of leaves of each plant were subjected to various physicochemical evaluations viz. (color, odor, nature, %yield, test for alkaloids, glycosides, flavonoids, tannins etc.)

Microorganisms used:

Fungi: *Candida albicans*, *Mucor indicus* and *Aspergillus flavus* (ATCC).



Evaluation of antifungal activity: The *in-vitro* antifungal activity of chloroform, alcohol and aqueous extracts of leaves of *Achyranthes aspera* and *Plectranthus amboenicus* of different concentrations (25, 50 and 100 µg/ml) was evaluated by disc plate method, using Sabouraud's broth medium. Fluconazole (5 µg/ml), was used as reference standard drug for comparative study^{8,1}.


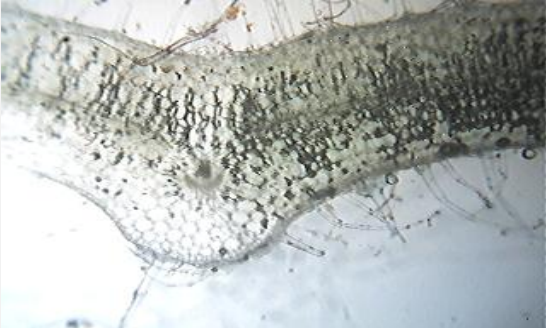
Preparation and evaluation of antifungal activity of polyherbal formulation: The polyherbal formulation was prepared by using the concentration (µg/ml) at which the respective extracts showed zone of inhibition. The homogenous mixture of the extracts was prepared by using a suitable pharmaceutical base and the antifungal activity of the polyherbal formulation was evaluated.

RESULTS:

Pharmacognostic study: (Table 1)

TABLE 1: MICROSCOPIC AND MACROSCOPIC STUDY OF LEAVES OF ACHYRANTHES ASPERA AND PLECTRANTHUS AMBOENICUS

Sr. no	Parameters	<i>Achyranthes aspera</i> leaf	<i>Plectranthus amboenicus</i> leaf
01	Macroscopy		

	a) Color	Green	Green
	b) Size & Shape	Leaves are petiolate, alternate, elliptic- obovate and acute, it is 5 cm long and 3 cm broad.	Leaves sinuate-toothed, lower large, 15-30 cm long, oblong-ovate or rounded or palmately 3-5 lobed, upper small 5-8 long, lanceolate, petioles with a pair of stipular glands.
2	Microscopy	<p>a) Trichomes: - It showed presence of lignified multicellular trichomes.</p> <p>b) Palisade cells: - The palisade cells are elongated and single layered</p> <p>c) Vascular bundle: - The vascular bundle showed the presence of xylem and phloem.</p> <p>d) The entire T.S has showed the presence of collenchymas.</p> <p>e) Starch grains are present.</p> 	<p>a) Trichomes: - It showed presence of lignified multicellular trichomes.</p> <p>b) Palisade cells: - The palisade cells are elongated and single layered</p> <p>c) Vascular bundle: - The vascular bundle showed the presence of xylem and phloem.</p> <p>d) The entire T.S has showed the presence of collenchymas.</p> <p>e) Starch grains are present.</p> 

Physicochemical evaluation of leaves of each plant: (Tables 2 - 10)

TABLE 2: PHYSICOCHEMICAL EVALUATION OF LEAF POWDER OF *ACHYRANTHES ASPERA*

Sl. No.	Parameters	Observation
I	Ash values:	
	Total ash	10.2 % w/w
	Acid insoluble ash	0.6 % w/w
II	Extractive value:	
	Methanol soluble	2.5 % w/w
III	Loss on drying	52.0 % w/w

TABLE 3: PHYSICOCHEMICAL EVALUATION OF LEAF POWDER OF *PLECTRANTHUS AMBOENICUS*

Sr. No.	Parameters	Observation
I	Ash values:	
	Total ash	12.5% w/w
	Acid insoluble ash	0.9% w/w
II	Extractive value:	
	Methanol soluble	1.6% w/w
III	Loss on drying	60.0% w/w

Physicochemical evaluation of extracts:

TABLE 4: PHYSICAL EVALUATION OF *ACHYRANTHES ASPERA* LEAF EXTRACTS

Sr. no.	Evaluation of <i>Achyranthes aspera</i> leaf extracts		
Parameters	Chloroform	Alcohol	Aqueous
Color	Light green	Dark green	Reddish brown
Nature	Semi Solid	Semi solid	Solid
Percentage yield	8.33 % w/w	14 % w/w	20 % w/w

TABLE 5: PHYSICAL EVALUATION OF *PLECTRANTHUS AMBOENICUS* LEAF EXTRACTS

Sr. no.	Evaluation of <i>Plectranthus amboenicus</i> leaf extracts		
Parameters	Chloroform	Alcohol	Aqueous
Color	Light green	Dark green	Reddish brown
Nature	Semi Solid	Semi solid	Solid
Percentage yield	10 % w/w	16.66 % w/w	21.66 % w/w

TABLE 6: QUALITATIVE CHEMICAL ANALYSIS OF *ACHYRANTHES ASPERA*

Sr. no	Qualitative Chemical Analysis of <i>Achyranthes Aspera</i> Leaf Extracts		
Parameters	Chloroform	Alcohol	Aqueous
Test for Alkaloids	+	+	-
Test for Glycosides	+	+	+
Test for Tannins	-	+	+
Test for Saponins	-	+	+
Test for Flavonoids	-	+	+

TABLE 7: QUALITATIVE CHEMICAL ANALYSIS OF *PLECTRANTHUS AMBOENICUS*

Sr. no.	Qualitative Chemical Analysis of <i>Plectranthus amboenicus</i> Leaf Extracts		
Parameters	Chloroform	Alcohol	Aqueous
Test for Alkaloids	+	+	-
Test for Glycosides	-	-	+
Test for Tannins	-	+	+
Test for Saponins	+	+	-
Test for Flavonoids	-	+	+

TABLE 8: ANTIFUNGAL ACTIVITY OF *ACHYRANTHES ASPERA* LEAF EXTRACTS

Sr. no	Name of fungi	Zone of inhibition in mm. of <i>Achyranthes aspera</i> leaf extracts ($\mu\text{g/ml}$)									
		Chloroform extract			Alcohol extract			Aqueous extract			Fluconazole
		25	50	100	25	50	100	25	50	100	5 $\mu\text{g/ml}$
01	<i>Candida albicans</i>	-	-	+	-	+	+	-	+	+	+
02	<i>Mucor indicus</i>	-	-	+	-	+	+	-	+	+	+
03	<i>Aspergillus flavus</i>	-	-	+	-	+	+	-	+	+	+

TABLE 9: ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS *PLECTRANTHUS AMBOENICUS*

Sr. no	Name of fungi	Zone of inhibition in mm. of leaf extracts <i>Plectranthus amboenicus</i> ($\mu\text{g/ml}$)									
		Chloroform extract			Alcohol extract			Aqueous extract			Fluconazole
		25	50	100	25	50	100	25	50	100	5 $\mu\text{g/ml}$
01	<i>Candida albicans</i>	-	-	+	-	+	+	-	+	+	+
02	<i>Mucor indicus</i>	-	-	+	-	+	+	-	+	+	+
03	<i>Aspergillus flavus</i>	-	-	+	-	+	+	-	+	+	+

TABLE 10: ANTIFUNGAL ACTIVITY OF POLYHERBAL FORMULATION

Sr. no	Name of fungi	Zone of inhibition in mm. of <i>polyherbal formulation</i> ($\mu\text{g/ml}$)									
		Chloroform extract			Alcohol extract			Aqueous extract			Fluconazole
		25	50	100	25	50	100	25	50	100	5 $\mu\text{g/ml}$
01	<i>Candida albicans</i>	-	-	+	+	+	+	-	+	+	+
02	<i>Mucor indicus</i>	-	-	+	+	+	+	-	+	+	+
03	<i>Aspergillus flavus</i>	-	-	+	+	+	+	-	+	+	+

DISCUSSION: In the present study, an attempt was made to investigate a detailed pharmacognostic, physicochemical and antifungal activity of leaf extracts of *Plectranthus amboenicus* and *Achyranthes aspera*.

The results revealed that the alcoholic and aqueous extracts of both the plant materials have shown a significant antifungal activity at different concentrations which was comparable to that of standard drug flucanazole (5µg/ml). The chloroform extracts of leaves of *Plectranthus amboenicus* and *Achyranthes aspera* have elicited less significant activity comparable to that of standard drug.

Furthermore, the polyherbal formulation was prepared by using a suitable pharmaceutical base and the active concentration (100 µg/ml) of alcoholic and aqueous extracts of leaves of *Plectranthus amboenicus* and *Achyranthes aspera*. The polyherbal formulation was evaluated for its antifungal activity and it was found that the polyherbal formulation have elicited promising antifungal activity which was comparable with that of standard drug. However the activity was due to the synergetic blend of herbal drugs. Subsequently the polyherbal formulation will be evaluated for various pharmaceutical quality control tests to assure its stability, safety, efficacy and acceptability.

Hence, to put in nutshell the leaves of *Plectranthus amboenicus* and *Achyranthes aspera* particularly alcoholic and aqueous extract possessed good antifungal activity.

However, this claims demand for further studies and isolation of the active phytoconstituents of individual components and observing their effect as a promising antifungal agent.

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