

ANTI-DANDRUFF ACTIVITY OF *RICINUSCOMMUNIS* L. LEAF EXTRACTS

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## ABSTRACT

*Ricinuscommunis* L. leaves were extracted with various solvents for its efficacy against *Malasseziaspp*, causative agent of dandruff in people who have over active sebaceous glands. Dandruff samples were collected and inoculated in various media formulations supplemented with coconut oil, tween 80 and olive oil to determine the optimized conditions for the isolates. Phytochemical analysis of the leaf extracts revealed the presence of flavonoids, saponins, tannins, phlobatannins and terpenoids as major phytoconstituents. Among the media formulations, potato dextrose agar supplemented with coconut oil has recorded better growth of the isolates. Various degree of inhibition by the leaf extracts was observed throughout the study. Significant inhibitory action with methanol extract was recorded against *Malasseziasp* which was mainly influenced by flavonoids, saponins and tannins followed by aqueous extract. Moderate activity of chloroform and petroleum ether extracts were observed due to the absence of major phytochemicals revealing the role of these compounds in controlling the growth of *Malassezia* sp. Utilization of natural products especially plants as medicines is one way to overcome the illness caused by microorganisms and the present study concluded that *R. communis* could be one among them to control dandruff.

**Keywords:** *Malassezia*, Antidandruff, *Ricinuscommunis*, Phytochemicals

## INTRODUCTION

Dandruff is a chronic scalp condition characterized by visible flakes induced by rapid turnover of scalp cells. In general, dandruff occurs after puberty and mainly affects males more than the females<sup>1</sup>. Dandruff results from at least three etiologic factors: *Malassezia* fungi, sebaceous secretions, and individual sensitivity<sup>2, 3</sup>. *Malasseziaspp* are involved in the etiology of pityriasisversicolor, folliculitis, seborrheic dermatitis and dandruff<sup>4,5</sup>. They are normally found in areas rich in sebaceous glands as they are lipid dependent.

The genus *Malassezia* belongs to the basidiomycetous yeasts and is classified in the Malasseziales (Ustilaginomycetes, Basidiomycota)<sup>6,7</sup>. Fungi in the genus *Malassezia* are ubiquitous skin residents of humans and other warm-blooded animals. *Malassezia* are involved in disorders including dandruff and seborrheic dermatitis, which together affect >50% of humans<sup>8</sup>.

*Ricinuscommunis*L. (Euphorbiaceae) grows as weed in the tropical warm regions. Various parts of the plant have been used to treat liver disorders, hypoglycemic conditions<sup>9-11</sup> and exhibited anti-inflammatory, antinociceptive, antioxidant and cytotoxic activities<sup>12-17</sup>.

Most of the antifungal agents available are inducing resistant development among the pathogens or producing adverse side effects hence there is a need of new antifungal agents lacking those disadvantages and plant secondary metabolites are in consensus to produce potential drugs. The present study was undertaken to investigate and determine the antidandruff activity of various solvent extracts of *R. communis* leaves.

## MATERIALS AND METHODS

## Plant material collection and extraction

Leaves of *Ricinuscommunis* were collected, dried under shade for 72 hours, ground into powder and passed through 60 mesh. About 20 g of the powder was extracted with 200 ml each of distilled water, chloroform, methanol and petroleum ether for 48 hours at room temperature. The extracts were filtered through Whatman No.1 filter paper and concentrated to dryness in rotary evaporator under reduced pressure.

## Phytochemical analysis

The extracts were tested for the presence of active chemical compounds by following the methods described by Trease and Evans (1989)<sup>18</sup>.

## Test for Tannins:

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

## Test for Phlobatanins

1% of HCl was added to the extract (1ml) and boiled in hot water bath. Formation of red precipitate indicates the presence of phlobatannins.

## Test for Saponins

1 ml of the extract was taken in a test tube and distilled water (2ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of Froth formation persisted for next one hour confirms the presence of saponins.

## Test for Flavonoids

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

## Test for Terpenoids

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of conc. sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

## Test for Cardiac Glycosides

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of 2 ml of glacial acetic acid, 1 drop of ferric chloride solution and 1 ml of conc. sulphuric acid. Formation of brown ring at interface confirms the presence of cardiac glycosides.

## Isolation of dandruff causing agent

Samples were collected by scraping the lesions of patients and stored in sterile containers in refrigerator until used. Different media formulations (Dixon agar, potato dextrose agar, Sabouraud's agar) were supplemented with coconut oil, tween 80, olive oil and inoculated with the sample. The plates were incubated at 37°C for 3-5 days. After incubation, the colonies were stained with Loeffler's

methylene blue and catalase test was performed for the identification of the causative agent.

#### Antidandruff assay

Various concentrations of the extracts were prepared in DMSO from the resultant extract to determine its antidandruff activity. Control experiments were performed by using DMSO with identical concentration used to test the extract. Isolates from dandruff were inoculated by swabbing on the surface of gelled media plates. Wells of 6 mm in diameter were performed in the PDA media, and each well was filled with 50  $\mu$ l of certain concentration of extract. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the extract and thereafter incubated at 37°C for 3-5

days. The radius for the zone of inhibition was measured in two directions at right angles to each other by measuring in millimeters and recorded against the corresponding concentration. Experiments were carried out with three replicates per treatment and the data obtained were statistically analyzed using Analysis of Variance (ANOVA).

#### RESULTS AND DISCUSSION

Among the various media formulations, potato dextrose agar supplemented with coconut oil exhibited significant growth after 5 days at 37°C followed by Dixon agar with tween 80. Loeffler's methylene blue staining of the colonies appeared as budding yeast cells and strong positive catalase reaction by the isolates was observed.

**Table 1: Phytochemical analysis of *R.communis* leaf extracts**

Phytochemicals	Aqueous	Methanol	Chloroform	Petroleum ether
Alkaloids	-	+	-	-
Anthroquinones	-	-	-	-
Flavonoids	+	+	-	-
Glycosides	-	-	-	-
Phenolics	-	-	-	-
Phlobatannins	-	+	-	-
Saponins	+	+	-	-
Steroids	-	-	-	+
Tannins	+	+	+	+
Terpenoids	+	+	+	-

+ = presence; - = absence

The present investigation revealed that the *R.communis* leaf extracts has antidandruff effects and due to its potential bioactive compounds. Phytochemical screening of various solvent extracts of *R.communis* leaves revealed the presence of flavonoids, saponins, tannins, phlobatannins and terpenoids (table-1). The effect of different extracts at various concentrations of *R.communis* growth of *Malassezia* sp is presented in table - 2. The results shows that aqueous and solvent extracts significantly varied in antidandruff activity which may be attributed by the nature of phytochemicals and their relative solubility in corresponding solvents. Among the

various solvents used, methanolic extracts exhibited significant activity ( $8.20 \pm 0.3$ ) which was attributed by the solubility of bioactive compounds and stronger extraction capability of the solvent. Aqueous extracts of the leaves recorded appreciable inhibitory activity ( $5.74 \pm 0.8$ ) when compared with chloroform ( $1.66 \pm 1.2$ ) and petroleum ether extracts. Zone of inhibition was increased with higher concentrations of the extracts revealing the role of phytochemicals against the isolates. Petroleum ether extract gave a mean zone of inhibition of  $0.90 \pm 0.3$  mm, showing the ineffectiveness of the extract.

**Table 2: Mean zone of inhibition in mm**

Extract	Concentration ( $\mu$ g/ml)				
	100	200	300	400	500
Aqueous	0.0	$0.34 \pm 0.1$	$1.20 \pm 0.8$	$3.15 \pm 1.1$	$5.74 \pm 0.8$
Chloroform	0.0	0.0	$0.60 \pm 0.9$	$1.65 \pm 0.7$	$1.66 \pm 1.2$
Methanol	0.0	$1.58 \pm 0.9$	$6.90 \pm 0.6$	$7.47 \pm 1.4$	$8.20 \pm 0.3$
Petroleum ether	0.0	0.0	0.0	$0.20 \pm 0.0$	$0.90 \pm 0.3$

Processing of plant materials, methods of extraction and solvents used can influence the activities of the bio active molecules from plants and thus various degree of inhibition were observed in the study. These secondary plant metabolites exert antimicrobial activities through different mechanisms. Antimicrobial properties of flavonoids have been well documented<sup>19</sup> and flavonoids were present as the major phytochemical in the present investigation which could be influenced the antidandruff activity of the leaves. The human skin-associated *Malassezia* spp requires lipid for its growth<sup>20, 21</sup> and lipase activities of the genus has been reported<sup>22</sup>. One of the antimicrobial activities of tannins is by inhibiting extracellular enzymes<sup>23, 24</sup> and the presence of tannins in the solvent extracts could have interfered with the lipase activity of the isolates in the present study. Interactions with membrane sterols lead to the antimicrobial activity of saponins and its presence in aqueous and methanol extracts influenced the antidandruff activity of *R.communis*. Antidandruff activities of *Hibiscus rosasinensis*<sup>25</sup>, *Tridax procumbens*<sup>26</sup> and *Trigonella foenum*<sup>27</sup> were reported in previous studies.

Different combinations of solvents possibly extract newer compounds but the use of solvents in diluted form or aqueous extracts in particular reduce the solvent usage and paves way for the green technology. Though some of the bio active compounds are soluble only in high polar solvents as in the present study, modifications in

extraction procedures like temperature, time and fractionations could efficiently extract the phytochemicals. In the present investigation, methanolic extracts have exhibited significant antidandruff activity and aqueous extracts also exhibited appreciable activity than the chloroform and petroleum ether extracts proving that it can reduce the use of other organic solvents to extract the phytochemicals. However, standardization of extraction by using aqueous based solvents is necessary for the minimal use of solvents.

Though a variety of antifungal drugs are available, their usage is restricted either its fungistatic action or expensive nature and phytochemicals are less potent anti-infectives than the antibiotics<sup>28</sup>. Hence an herbal drug is need of the hour for the control of dandruff and *Ricinus communis* has been proven one among them in the present study. It is concluded that *R.communis* leaves could be a potential source of active antidandruff agents and its *in vivo* potencies and toxicological profile to be investigated in order to develop new bioactive principles.

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