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Research Article

BASIC PHYTOCHEMICAL STUDY AND EVALUATION OF ANTIDANDRUFF POTENTIAL OF FRUIT JUICES OF CITRUS LIMON. L

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This study investigates the scientific proof for phytochemical composition and also the proof of antidandruff activity. Screening of phytoconstituents was carried out on the juice concentrates using established procedures. The anti-dandruff activity was carried out by Agar Well Diffusion Assay method against *Malassezia furfur*, which is the organism responsible for dandruff. The result of antidandruff activity revealed that juice concentrates of the fruits of *citrus lemon* (10 mg extract/mL) exhibited highly significant antifungal activity against *Malassezia furfur*. The results of Minimum inhibitory concentration (MIC) study reveal that the extract possesses anti-dandruff activity above the concentration of 0.1%w/v. The results of MIC study revealed that the extract possesses anti-dandruff activity in a concentration-dependent manner against the test organism and were comparable to the standard drug.

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sreekanthsk16@yahoo.co.in**Funding:** Nil**Competing Interests:** Nil**Keywords:** Antidandruff, *Citrus limon*, *Malassezia furfur*.**INTRODUCTION:**

The Father of Medicine-Hippocrates said, let your food be your medicine, who compiled a list of over 400 herbs and its uses. Over many centuries, our experiment with plants has yielded a vast stock of natural medicines to help us to heal many ailments, without any harmful side effects [1].

Citrus limon, lemon, is a small tree in the family of Rutaceae in the genus citrus. This plant was originated in Asia and is now grown commercially worldwide in tropical and semi-tropical countries, including the Mediterranean region. The fresh fruit is used in beverages and for cooking.

The lemon tree grows to 6 m height and it has stout spines. The leaves are dark green in color, leathery and evergreen, oblong, elliptical, or oval in shape and up to 14 cm long. Flower buds are purplish, but flowers open to have 5 white petals. Fruits are globule to oblong in shape, 7.5 to 12.5 cm long, and yellow when ripens, with smooth peels dotted with oil glands. Lemon fruits are highly acidic and are rich in citric acid and vitamin C. Their tart flavor is popular in beverages, ice creams, desserts, salad dressings, and many meat and vegetable dishes. Lemons have antioxidant properties [2], so lemon juice is often added to fresh fruit to prevent oxidation and browning. Lemon peel or zest (the outer peel) is used as a flavoring. Lemon oil, obtained from the peel, is used as a wood cleaner and polish, and as a non-toxic pesticide. Traditional medicinal uses for the fruit,

peels, oil, and the oil obtained from the seeds include treating fever and colic, and as an astringent and diuretic effects.

Dandruff is a common scalp condition and it is produced when the skin of the scalp exfoliates extremely and also produces an itchy sensation on the scalp. Dandruff is related to a fungus known as *Malassezia* that lives on the scalp of the people. In some cases, overgrowth of the fungus results in the flakiness of the skin characteristic of dandruff [3]. To conduct systematic phytochemical screening in citrus *limon* fruits and also to conduct the antidandruff potential activity of the juice concentrate of citrus *limon* fruits.

MATERIALS AND METHOD

Collection and Identification of Plant Samples: Fresh lemon fruits were purchased from the vegetable market, Ad Dawadimi. The fruits were identified by the supervisor of the research.

Preparation of fruit juice concentrates:

The fruits were washed thoroughly with distilled water and cut it into halves and the juice was extracted from the fruits using a juice extractor. The collected fruit juices were then lyophilized and the concentrates were preserved at 4°C in airtight containers until subsequent use.

Screening of Phytoconstituents: Screening of phytoconstituents was carried out on the juice concentrates using established procedures [4, 5].

The concentration of the stock solution used for the phytochemical screening was 10 mg extract/mL distilled water. Various phytoconstituents like alkaloids, flavonoids, steroids, terpenoids, reducing sugar, saponins and cardiac glycosides in the juice concentrates were analyzed. All the chemicals and reagents used were of analytical grade.

Test for Carbohydrates

Molisch's Test: Treat 1mL of test solution with few drops of alcoholic α -naphthol. Add 0.2 mL of concentrated sulphuric acid gently along the sides of the test tube. Purple to violet colour ring appears at the junction of the solution.

Fehling's Test: 1mL of Fehling's A and B were mixed and added few drops of sample and boiled. A brick red precipitate of cuprous oxide will form, which shows the presence of reducing sugar [4, 5].

Test for Alkaloids

Mayer's Test: Alkaloids will give a cream color precipitate when treated with Mayer's reagent [4, 5].

Dragendorff's Test: Alkaloids will produce reddish brown precipitate with Dragendorff's reagent [4, 5].

Test for Sterols and Terpenoids

Libermann-Burchard Test: The prepared extract was treated with a few ml of acetic anhydride, boil and cool, concentrated sulphuric acid is added on the sides of the test tube, presence of brown ring at the junction of two layers and the top layer will changes to green which shows the presence of sterols. If terpenoids is present, it will show deep red colour.

Salkowski's Test: Treat the prepared extract in chloroform with a few ml of concentrated sulphuric acid, shake well and allows it to stand for some time, if the red colour appears in the lower layer, indicates the presence of sterols and formation of yellow colour in the lower layer shows the presence of terpenoids.

Test for Glycoside

Keller Killiani Test (Cardiac glycosides): To little of the plant extract, add little mL of glacial acetic acid containing a trace amount of ferric chloride. In a small test tube; add carefully 0.5 mL of Conc. sulphuric acid along the sides of the test tube. Blue colour appears in the acetic acid layer, shows the presence of cardiac glycosides [4, 5].

Borntrager's Test (Anthraquinone Glycosides): Little mL of the plant extract was treated with benzene and the organic layer was separated and half of its own volume of 10% ammonia solution was added. A pink, red or violet colour in the ammonical phase indicates that of anthraquinone glycosides [4, 5].

Test for Glycoside

Forth Test: A small amount of the dried powdered plant was added to few mL of distilled water. Then it is shaken firmly. The form indicates saponin presence.

Test for Flavanodis

Shinoda Test (Magnisium hydrochloride reduction test):

Into the test solution, added little magnesium ribbon and add concentrated hydrochloric acid drop by drop. A pink, scarlet-crimson red or green-blue colour appears after few a minutes.

Determination of antidandruff activity

Agar Well Diffusion Assay [6]

The antidandruff activity assay was carried out by Agar Well Diffusion Assay method at concentrations of 0.1%w/v, 0.2%w/v, and 0.3%w/v. Pure culture of *M. furfur* Petri plates containing 20mL of Sabouraud dextrose agar media were inoculated with 100 μ L of diluted cultures using spread plate technique and were allowed to dry in a sterile chamber. 5mm wells were cut using a cork borer on the surface of the inoculated agar. The plant extracts were filtered and sterilized using a 25mm syringe filter, loaded into wells and were allowed to dry completely. The antidandruff activity was assessed by measuring the inhibition zone. Clotrimazole was used as the positive control.

Statistical analysis

Data were analyzed using Microsoft excel. All tests were performed in triplicate and data are expressed as Mean $\pm$ SD (standard deviation).

RESULT AND DISCUSSION

Phytochemicals are plant chemicals, possessing varying degrees of disease preventive properties. They are invaluable sources of basic drug materials for both traditional and modern medicine. In this study, the phytochemical composition of the citrus juice concentrates showed the presence of flavonoids, terpenoids, glycosides, and reducing sugars (Table 1).

These results agree at par with the findings of other studies [7, 8] who reported the presence of reducing sugars, flavonoids as antioxidants, and terpenoids in lemon juice. However, they did not detect the presence of alkaloids, saponins, and glycosides, but steroids were present in lemon. These differences may be due to differences in species and geographical location.

The results of antidandruff activity revealed that juice concentrates of the fruits of Citrus limon (10 mg extract/mL) exhibited highly significant antifungal activity against *Malassezia furfur*. The results of MIC study revealed that the extract possesses antidandruff activity at and above the concentration of 0.1%. The results of zone of inhibition study revealed that the extract possesses antidandruff activity in a concentration dependent manner against the test organism and were comparable to the standard drug. The results obtained from the tests were as shown below:

Phytochemical	Present (+) Absent (-)
Alkaloids	-
Flavonoids	+
Steroids	+
Terpenoids	+
Reducing sugar	+
Saponins	-
Cardiac glycosides	-

Table 1: Phytochemical composition of citrus limon fruit juice concentrates

TEST ORGA NISM	ZONE OF INHIBITION (cm $\pm$ SD, N=3)			
	Juice concentrates of the fruits of Citrus limon (10 mg extract/mL)			Std. Clotrimazole (0.1% w/v)
	0.1%w/v	0.2%w/v	0.3%w/v	
M. furfur	10 $\pm$ 006	11 $\pm$ 001	15 $\pm$ 006	17 $\pm$ 001

Table 2: Agar Well Diffusion Assay

CONCLUSION

Most of the world population is looking for the treatment of dandruff. This study proves that a preparation containing more than 0.1% w/v of Juice concentrates of the fruits of Citrus limon (10 mg extract/mL) will inhibit the *Malassezia furfur*, which is the responsible micro organism for dandruff, and thus reduces the amount of dandruff. Thus, the objective of the study was achieved. The results of this study have revealed that these commonly consumed citrus juices may contain promising antidandruff leads. This study, however, provides *in-vitro* data which may be replicated *in vivo*. Further studies directed at the isolation of novel antimicrobial compounds and *in vivo* studies that may validate the *in vitro* findings are recommended.

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