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ORIGINAL RESEARCH ARTICLE

Comparative Study of Antifungal Activity of Camellia *sinensis* and *Acacia sinuate Merr* against Dandruff Causing *Pityrosporum ovale*

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ABSTRACT

The medicinal value of *Camellia sinensis* and *Acacia sinuate Merr* were investigated through phytochemical study and *in vitro* antifungal activity of their various extracts (petroleum ether, chloroform, ethanol and water). The analysis of extracts of the two plants revealed the presence of carbohydrates, saponins, flavanoids, tannins and phenolic compounds. The antifungal activity of *Camellia sinensis* and *Acacia sinuate Merr* was tested against dandruff causing *Pityrosporum ovale* at concentrations of 10, 5 and 2.5 mg/ml assayed by Filter paper disc diffusion technique. The active components present in petroleum ether and ethanolic extracts of *Camellia sinensis* showed MIC against *P. ovale* compared to *Acacia sinuate Merr* in treating dandruff.

Key words: *Camellia sinensis, Acacia sinuate Merr,* phytochemicals, antifungal activity, *Pityrosporumovale*

INTRODUCTION

Dandruff is a common embracing scalp disorder affecting a large chunk of population. The yeast like fungus *Pityrosporum ovale* is the causative microorganism of dandruff and due to its lipase activity it releases pro inflammatory free fatty acids, causing dermal inflammation and tissue damage [1]. Plants containing flavanoids, terpenoids, steroids, and phenolic compounds have been reported to have antimicrobial activity [2].

Camellia sinensis (Green tea) commonly called as Teyillai in Tamil language belongs to the family Theaceae. This plant has been traditionally useful in treating inflammations, asthma, and heart diseases, lowering blood sugar and fighting cancer. It is also useful in wound ulcers, coughs, bronchitis, burning sensation, diarrhoea, dysentery, leprosy, fever, hair fall, greyness of hair and various skin diseases [3]

Acacia sinuate Merr (Soap nut) commonly called as Cikkai in Tamil language, belongs to the family Mimosaceae. This plant is useful as astringent, cooling, emetic, detergent, depurative and antihelminthic. They are useful in vitiated condition of pitta, burning sensation, constipation, renal and vesical calculi, haemorrhoids, erysipelas, leucoderma, leprosy, prurigo, abscesses and eczema. The powerful pods are the best alternative to soaps in all cases of skin diseases [4].

In order to authenticate the traditional medicinal claims, the present study was carried out to compare and evaluate the antifungal activity of *Camellia sinensis* and *Acacia sinuate Merr* plant extracts.

MATERIALS AND METHODS

Camellia sinensis (Green tea) and *Acacia sinuate Merr* (Soap nut) were collected from Navamalai region of Pollachi taluk in Coimbatore district and were authenticated in TamilNadu Agricultural University

The plant materials were shade dried and powdered. About 50gm of air dried plant materials were extracted with 250 ml of various solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water) using Soxhlet apparatus for 72 h at 31^0 C [5]. The extracts were concentrated to dryness. The crude extracts were then used for the evaluation of phytochemical components and antifungal activity against *Pityrosporum ovale*.

Phytochemical Screening

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Conventional standard protocols [6] for detecting the presence of different chemical constituents in the plant extract were employed. The tests for alkaloids, tannin, saponins, glycosides, carbohydrates, flavanoids and phenols were carried out. Phytochemical screening of plants was carried out to detect the bioactive compounds using qualitative test [7]. The extracts obtained were subjected to qualitative tests for identification of chemical constituents of selected parts of the plant [8].

Antifungal Activity

In vitro screening for antifungal study was carried out using dandruff causing *Pityrosporum ovale*. It was identified by following the standard microbiological methods [9]. The antifungal screening of extracts were carried out by determining the zone of inhibition using Kirby Bauer disc diffusion method [9]. The filter paper disc diffusion method was used for antibiotic susceptibility testing [9] using different concentration of 10 mg/ml, 5mg/ml and 2.5mg/ml of plant extracts dissolved in dimethyl sulphoxide. To perform the filter paper disc diffusion method, the fungal strains was grown in Sabourand's dextrose broth and incubated at 30^0 C fro 24 hours. Sterile Sabourand's dextrose plates were prepared and a lawn culturing of *Pityrosporum ovale* was done. The impregnated discs with plant extracts were placed on the agar surface aseptically. The plates were incubated at 30^0 C for 24-48 hours. After incubation, the zone of inhibition was measured [10].

The fungicidal activity of *Camellia sinensis and Acacia sinuate Merr* were found using broth dilution technique [11] The plant extracts were serially diluted and the dilutions were 8, 4, 2, 1, 0.5, 0.25 and 0.125mg/ml respectively. To each dilution, 0.1ml of 24 h fungal culture of *Pityrosporum ovale* was added. The tubes were incubated at 30^0C for 24 to 48h. The tubes were examined for visible turbidity. The minimum inhibitory concentration was determined. The MIC is the lowest concentration of the test compound inhibiting the appearance of growth of visible growth. If the MIC is more than 8mg/ml then the report mentions that the compound is active only at very high doses and the fractions need to be analyzed to reach the final conclusion. If the MIC is less than 8mg/ml the compound is considered active and worth following up [12].

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical analysis of various extracts of *Camellia sinensis* revealed the presence of flavanoids, glycosides, tannins, phenolic compounds, carbohydrates, amino acids and proteins. Extracts of *Acacia sinuate* showed the presence of saponins, glycosides and carbohydrates (**Table 1**). The presence of these bioactive compounds might be responsible for its antimicrobial properties. Though the body system is made in such a way that it tackles invading foreign substances in most cases, the body system is incapable to do so and needs to be protected, enhanced and activated. This ability to activate the body defense mechanism or to protect the body system has to be present in some herbal sources [13].

Table 1: Phytochemical analysis of *Camellia sinensis* and *Acacia sinuate* extracts

S. No	Bioactive components	*C. sinensis* extracts	*Acacia sinuate* extracts
1	Alkaloids	-	-
2	Steroids and sterols	-	-
3	Cardioglycosides	-	-
4	Flavanoids	+	-
5	Saponins	-	+
6	Glycosides	+	+
7	Tannins and Phenolic compounds	+	-
8	Terpenoids	-	-
9	Carbohydrates	+	+
10	Aminoacid and proteins	+	-

Antifungal Activity

The antifungal activity of *Camellia sinensis* and *Acacia sinuate Merr* extract were tested against *Pityrosporum ovale* at different concentrations using disc diffusion and well diffusion method using Kirby Bauer Disc diffusion method[10] (**Table 2 & 3**). Petroleum ether extracts of *Camellia sinensis* and *Acacia sinuate Merr* (10, 5, 2.5 mg/ml) exhibited zone of inhibition of 15, 13 & 10 and 14, 12 & 10 mm, respectively. Chloroform extracts of *Camellia sinensis* and *Acacia sinuate Merr* showed a zone inhibition of 12, 11& 8 and 8, 7 & 6 mm, respectively. Ethanolic extracts of *Camellia sinensis* and *Acacia sinuate Merr* showed inhibition zones of 13, 11& 9 and 12, 9& 7 mm, respectively.

Aqueous extracts of *Camellia sinensis* showed zones of inhibition of 9, 7& 6 mm and *Acacia sinuate Merr* didn't produce any zone of inhibition. Nalidixic acid and Co-trimoxazole was used as reference standards. The controls were prepared using the same solvents employed to dissolve the extracts. The inoculated plates with the test and standard discs were incubated at 37^0C for 24 h.

Table 2: Antifungal activity of *Camellia sinensis* on *Pityrosporum ovale* using Kirby BauerDisc Diffusion method.

S No	Extracts of *Camellia sinensis*	Zone of Inhibition (mm)		
		10mg/ml	5mg/ml	2.5mg/ml
1	Petroleum ether	15	13	10
2	Chloroform	12	11	8
3	Ethanol	13	11	9
4	Water	9	7	6

Table 3: Antifungal activity of *Acacia sinuate* on *Pityrosporum ovale* using Kirby Bauer Disc Diffusion Method

S No	Extracts of *Acacia sinuate*	Zone of Inhibition (mm)		
		10mg/ml	5mg/ml	2.5mg/ml
1	Petroleum ether	14	12	7
2	Chloroform	8	7	6
3	Ethanol	12	9	7
4	Water	No Zone	No Zone	No Zone

Table 4: The minimum inhibitory concentration of *Camellia sinensis* and *Acacia sinuate* extracts by Broth dilution method

S.No	Solvents and reference standards used	MIC of *Camellia sinensis* extracts (mg/ml)	MIC of *Acacia sinuate* extracts (mg/ml)
1	Petroleum ether	4.0	2.0
2	Chloroform	2.0	2.0
3	Ethanol	4.0	2.0
4	Water	2.0	No activity
5	Nalidixic acid	0.21	0.21
6	Co-trimoxazole	0.015	0.015

The minimum inhibitory activity of *Camellia sinensis* and *Acacia sinuate Merr* extracts were tested against *Pityrosporum ovale* using Broth dilution technique, and was less than 8mg/ml [11] (**Table 4**). The Nalidixic acid and Co-trimoxazole is used as the refernce standard. The MIC of petroleum ether and ethanolic extracts of *Camellia sinensis* is 4.0mg/ml, when compared to MIC of petroleum ether and ethanolic extracts of *Acacia sinuate* which is 2.0mg/ml. Chloroform extracts of *Camellia sinensis* and *Acacia sinuate* exhibited similar inhibitory concentrations. The MIC of aqueous extracts of No inhibition was shown by aqueous extracts of *Acacia sinuate,* when compared with *Camellia sinensis* which is 2.0 mg/ml.

The inhibition zones by disc diffusion method and MIC by the broth dilution method and revealed the antimicrobial activity of the extracts of Acacia species against bacterial and fungal strains [11].The antidermatophytic activity of methanol extract, freeand bound flavanoids of different parts of *Brassica campestris, Trigonella foenum gracecum*, *Piscum sativum* and *Camellia sinensis* against four dermatophyes in *Invitro* conditions [3]. A study on antifungal activity of lemon grass oil and lemon grass cream using broth dilution technique [15]. When compared to *Acacia sinuate* plant extracts, extracts of *Camellia sinensis* exhibited a greater inhibitory acivity on *Pityrosporum ovale*, as evidenced in the study.The results of various screening tests indicate some measurable inhibitory action of *Camellia sinensis* against *Pityrosporum ovale*, the dandruff causing yeast like fungus.

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