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RESEARCH ARTICLE

Antimicrobial activity of Antipsoriatic Plant *Givotia rottleriformis* Griff. Ex Wight

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ABSTRACT:

The plant *Givotia rottleriformis* have been used traditionally for treating skin diseases like psoriasis, for which there is no complete cure till date. *Psoriasis*, is a genetically determined chronic inflammatory dermatosis immuno disorder associated with over expression of proinflammatory cytokines characterized by red, scaly and raised patches that affects 2.3% of the population worldwide. According to American academy of dermatology, antibacterial therapy could also be used for treating psoriasis. In view of this, the present investigation was carried out to study the antibacterial activity with respect to their traditional use as antipsoriatic agents. The powdered bark was successively extracted with ethyl acetate and methanol and subjected to preliminary phytochemical analysis and HPTLC finger print analysis. The antimicrobial studies of ethyl acetate and methanol extract was carried out by agar diffusion method.

Among the ethyl acetate and methanol extract, methanol extract exhibited highest zone of inhibition at the concentration of 1000 ppm against *Staphyloccocus aureus* (18 mm) which has been one of the organisms which aggravate conditions like psoriasis, while moderate activity against *Streptococcus pyogenes* (12 mm). The zone of inhibition of ethyl acetate extract against *Escherichia coli* was 11 mm and against *Streptococcus pyogenes* was 14 mm at higher concentration (1000 ppm). The methanolic extract was ineffective against the fungal strain *Candida albicans* and ethyl acetate extract exhibited moderate effect (11 mm) against fungal strain at higher concentration (1000ppm). The minimum inhibitory concentration was also determined and found that the methanolic extract was required in relatively lesser quantities for arresting the growth of the tested organisms. The results obtained may provide validation for its reported medicinal uses.

KEYWORDS: *Givotia rottleriformis*, Psoriasis, antimicrobial, antibacterial, antipsoriatic herb.

INTRODUCTION:

Psoriasis is a common chronic inflammatory T-cell-mediated immune disorder characterized by circumscribed, red, thickened plaques with an overlying silver-white scale. Person of all ages may develop the disease. Clinically, psoriasis most frequently affects the skin of the elbow, knees, scalp, lumbosacral areas, intergluteal cleft and glans penis[1]. Psoriasis is a medical condition that occurs when skin cells grow too quickly. Faulty signals in the immune system cause new skin cells to form in days rather than weeks. The body does not shed these excess skin cells, so the cells pile up on the surface of the skin and lesions form[2]. The most typical lesion is a well demarcated, pink to salmon colored plaque covered by loosely adherent scales that are characteristically silver white in color.

The link between psoriasis and infection is probably explained by superantigen theory - superantigen is the products of bacteria, fungi or virus which can bypass normal immunological pathway and cause powerful stimulation to the immune system. Studies imply that a protein called M protein carried by *Staphylococcus*

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pyogens act as superantigen in provoking psoriasis[3]. Secondary infected dermatosis develops when a bacterium invades compromised skin such as psoriasis[4].

Bacteria play a key role in the exacerbation of psoriasis. Fungal infections, while commonly not systemic, can exacerbate psoriasis as well when the complication becomes systemic. Often, systemic fungal infections can be the most difficult to treat and lead to the greatest health risk especially in the psoriasis patient. The predominant aerobic and facultative bacteria were *S. aureus* (15 isolates), group *D. enterococcus* (2) and *Escherichia coli* (2). The predominant anaerobes were *Peptostreptococcus* spp. (6 isolates) and *Bacteroides* spp., *Propionibacterium acnes* and pigmented PREVOTELLA spp. in two each. Single bacterial isolates were obtained from 14 (61%) patients, 11 of which were S. AUREUS[5]. The most common form of psoriasis, for example, is related to streptococci in the throat, while the fungi *Candida* and *Malassezia*, in addition to *Staphylococcus aureus*, reside on the skin and are also associated with psoriasis. They propose that the lack of a normal skin barrier, the stratum corneum, might be one of the reasons why bacterial diversity differs in psoriasis. Apart from existing therapies, literature survey reveals that antibacterial therapy could be included for treating psoriasis[6] and many of the antibacterial agents, including those obtained from the herbal source are being used successfully for treating psoriasis.

Givotia rottleriformis Griff. Ex Wight is a moderately sized tree of the family Euphorbiaceae. The bark and seeds of the tree are used in indigenous medicine in the treatment of rheumatism, dandruff and psoriasis[7]. Previous studies have demonstrated that the Plant *Givotia rottleriformis* bark has antipsoriatic activity using *in-vivo* models[8]. These results prompted us to further investigate the species on the antibacterial activity with respect to their traditional use as antipsoriatic agents.

MATERIALS AND METHODS:

Dried bark of *Givotia rottleriformis* was powdered and passed through sieve #10. 30 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ethyl acetate and methanol successively. Before extraction with the next solvent, the powder was air dried to remove the adhering solvent. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven, and the dried extract was stored in air tight containers for further studies.

Preliminary Phytochemical Screening:

The ethyl acetate and methanol extract was subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins, phenols, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids[9].

High Performance Thin Layer Chromatography:

High performance thin layer chromatography was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20°C with Benzene: Methanol: Ammonia as a mobile phase in a CAMAG chamber previously saturated for 30 min. After development, the plate was dried at 60°C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software and the chromatograms were recorded[10].

Antibacterial activity assay

Procedure

The ethyl acetate and methanol extract were screened for antibacterial activity using the agar well diffusion method with sterile cork borer of size 6.0mm. The cultures of 24 hours old cultures, grown on nutrient broth are used for inoculation of bacterial strain on Muller Hinton agar plates. The extracts, after concentration, were weighed and dissolved in DMSO (1mg in 1ml). Each microorganism was diluted in sterile saline solution and adjusted to 0.1 OD reading. The above said microorganisms were then flooded on the surface of the pre sterilized Muller Hinton Agar plate. Two wells, each 10mm in diameter, were cut from the agar and 100 microliters (100ppm) of each extracts were loaded in to one well and antibiotic of same concentration was loaded into the other well. The plates were incubated for 24 hours at 37^{o}C. The complete antibacterial analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates[(11)].

Bacteria:

Staphylococcus aureus ATCC 9144, *Escherichia coli* ATCC 25922, *Streptococcus pyogenes* ATCC 12344 were used.

Fungi: *Candida albicans* ATCC 227 was used.

Minimal Inhibitory concentration:

The extracts were evaluated for their *in vitr*o growth inhibitory activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Candida albicans* Antimicrobial activities of the extracts were tested by the agar-diffusion method under standard

conditions. The bacteria was seeded into the nutrient broth and kept for incubation for 24 hours. A set of sterilized petri dishes was taken and to the petri dish, sterilized, molten Muller Hinton agar medium was poured and allowed to cool and solidified. After solidification, with a sterile cork borer, 6 wells are plucked. Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO). Further dilutions were performed with distilled water. The concentration range of the tested compounds was between 0.1 – 6 mg /ml. A control using DMSO without any test compound was included. The *MIC* values of the compounds to be tested were obtained as mg/ml. 50 microliters of each solution was placed in the well plucked in the pre- sterilized petridish. After incubation for 24 h at 25–27 °C, the diameters of the inhibition (sterile) zone (including disc) were measured (in mm). Every test was performed in triplicate.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening

Phytochemical analysis of the ethyl acetate extract shows the presence of carbohydrates, flavonoids, phenols, steroids, tannins, triterpenoids. and the methanolic extract shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, phenols, proteins, tannins, triterpenoids.

High Performance Thin Layer Chromatography

HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents. It is also a diagnostic method to find out the adulterants and to check the purity. The ethyl acetate and methanol extract were further subjected to HPTLC for the conformation of the active constituents. HPTLC was scanned at 254 nm with the best solvent to detect the maximum number of components and peak abundance qualitatively. The HPTLC chromatogram of the ethyl acetate and methanol extract of *G. rottleriformis* bark were shown in Figure 1 and 2. The chromatogram of ethyl acetate extract showed 5 spots in the solvent system with the R_f values 0.04, 0.32, 0.39, 0.70, 0.76 and methanol extract showed 8 spots in the solvent system with the R_f values 0.03, 0.20, 0.25, 0.27, 0.34, 0.61, 0.64, 0.73.

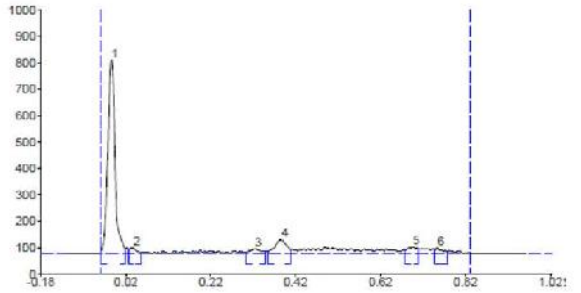

Figure 1: HPTLC fingerprint of ethylacetate extract of *Givotia rotterigormis* bark

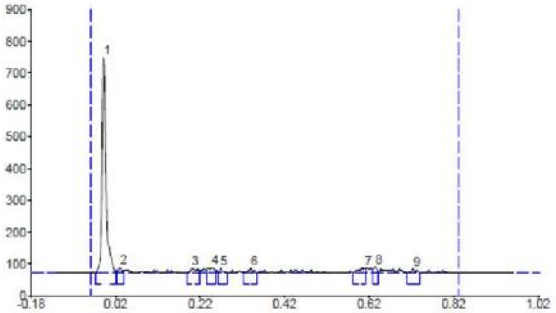

Figure 2: HPTLC fingerprint of methanol extract of *Givotia rotteriformis* bark

Antimicrobial screening:

The antimicrobial screening of the plant extracts were carried out by agar diffusion method. Among the tested extract, ethyl acetate and methanol extract showed increased number of phytoconstituents and both the extract were subjected to antibacterial and antifungal studies at different concentration (250ppm, 500ppm, 1000ppm). The organisms used were *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes* and *Candida albicans.* The results were tabulated in Table 1and 2.

Both the extract exhibited antibacterial activity against all bacterial strains. The results of both extracts in antibacterial study were comparable with that of the standard ciprofloxacin (30µg/ml /disc). Of all the tested concentrations of ethyl acetate extract and methanol extract, 1000ppm concentration was considered to be the most effective when compared to 250ppm, 500ppm concentration. In the present study, both methanolic extract and ethyl acetate extract was found to possess the broadest and potent antimicrobial activity against *S. aureus* and moderate active against the *E.coli* and *S. pyogenes* (Figure 3-6).

Table 1: Antimicrobial activity of ethyl acetate extract of *Givotia rottleriformis* bark

Ethyl acetate	Replicates	Microorganisms			
		S. aureus	***E. coli***	***S. pyogenes***	***Candida albicans***
250ppm	R1	10mm	9mm	6mm	No activity
	R2	10mm	10mm	8mm	No activity
	R3	9mm	10mm	8mm	No activity
500ppm	R1	12mm	9mm	11mm	No activity
	R2	11mm	8mm	10mm	No activity
	R3	11mm	8mm	13mm	No activity
1000ppm	R1	15mm	10mm	14mm	10mm
	R2	12mm	11mm	12mm	8mm
	R3	12mm	10mm	12mm	8mm
Antibiotic	R1	20mm	18mm	22mm	20mm

Values are mean ± SEM of 3 parallel measurements.

Table 2: Antimicrobial activity of methanol extract of *Givotia rottleriformis* bark

Methanol	Replicates	Microorganisms			
		S. aureus	***E. coli***	***S. pyogenes***	***C. albicans***
250ppm	R1	11mm	No activity	8mm	No activity
	R2	11mm	No activity	7mm	No activity
	R3	12mm	No activity	8mm	No activity
500ppm	R1	14mm	No activity	10mm	No activity
	R2	12mm	No activity	11mm	No activity
	R3	12mm	No activity	11.5mm	No activity
1000ppm	R1	18mm	08mm	12mm	No activity
	R2	16mm	09mm	10mm	No activity
	R3	16mm	08mm	11mm	No activity
Antibiotic	R1	20mm	18mm	22mm	20mm

Values are mean ± SEM of 3 parallel measurements.

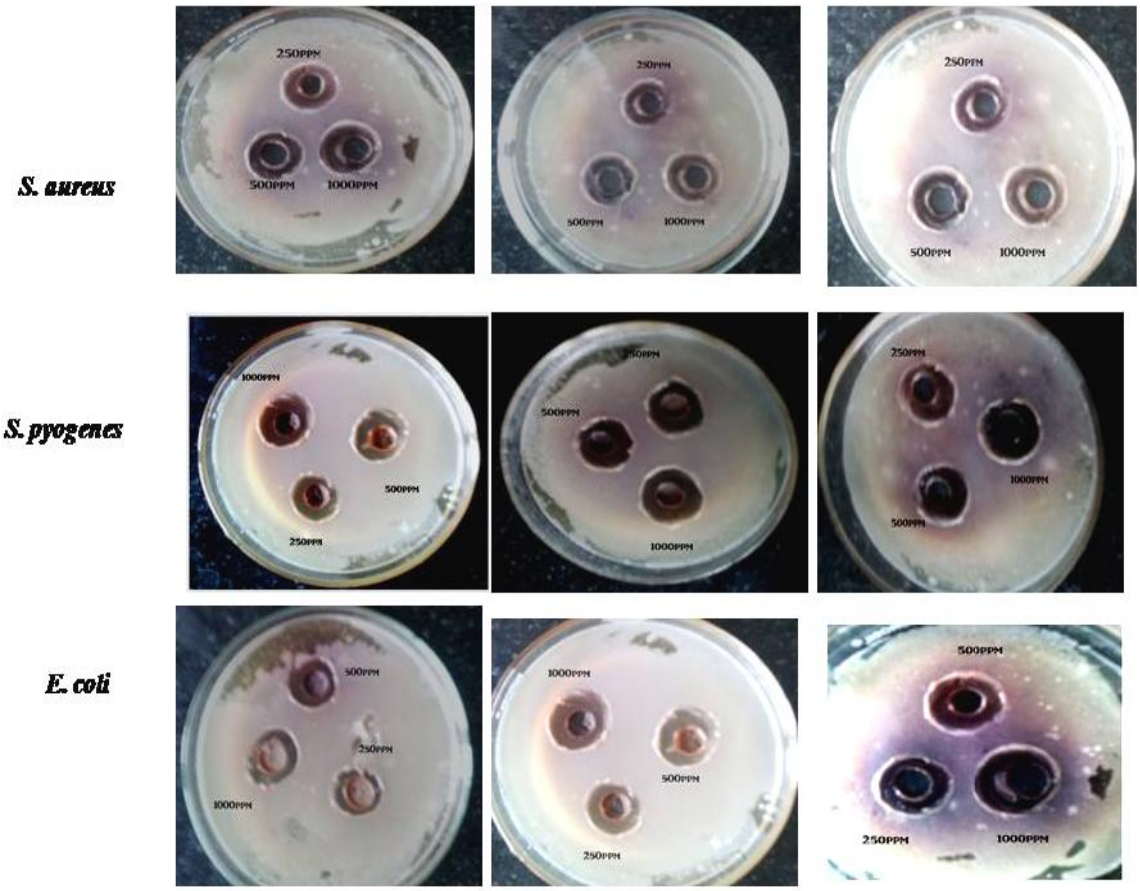

Figure 3: Inhibitory activity of Ethylacetate extracts of *Givotia rottleriformis* bark at 250, 500 and 1000 ppm concentration

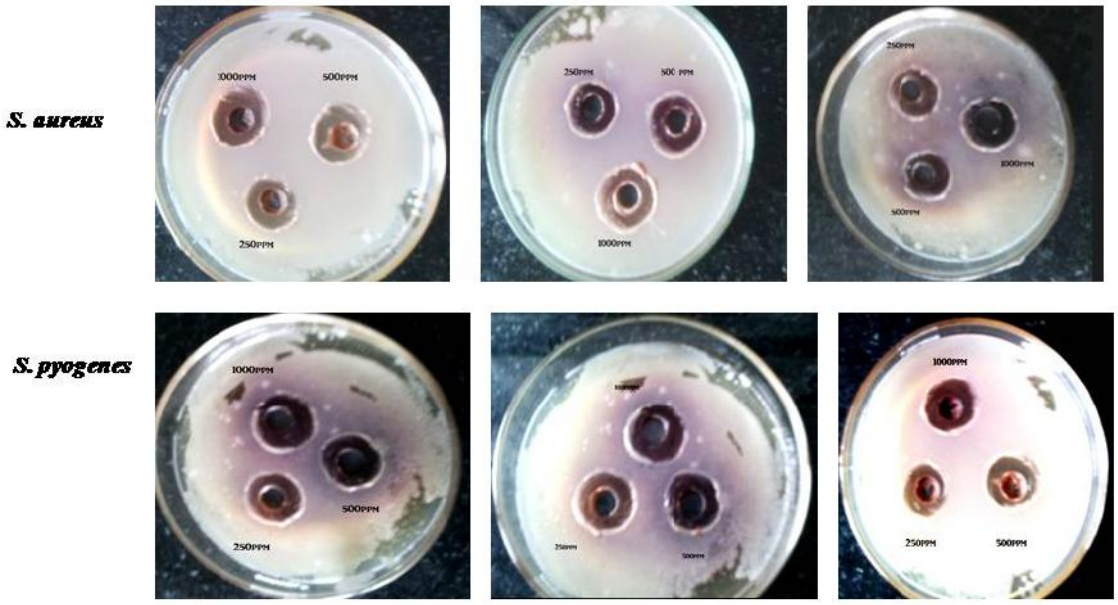

Figure 4: Inhibitory activity of methanol extract of *Givotia rotteriformis* bark at 250, 500 andf 1000 ppm concentration

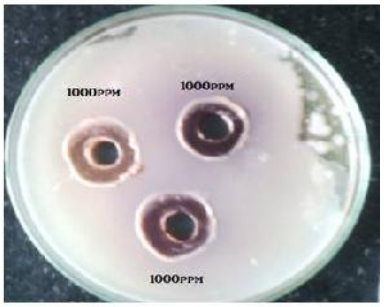

Figure 5: Inhibitory activity of Ethyl acetate extracts of *Givotia rottleriformis* bark against *Candida albicans* at 1000 ppm concentration

Figure 6: Inhibitory activity of Methanol extracts of *Givotia rottleriformis* bark against *Eschercia coli* at 1000 ppm concentration

The minimum Inhibitory Concentration:

The minimum inhibitory concentration was also determined. The MIC of ethyl acetate extract against *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* were 40, 66 and 52, mg/ml respectively and MIC of methanolic extract were 30 70 and 48 mg/ml respectively (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of ethyl acetate and methanol extract of *Givotia rottleriformis* bark

Organism		Minimum Inhibitory Concentration (mg/ml)	
		Ethyl acetate extract	Methanol extract
Bacteria	*Staphylococcus aureus*	40	30
	Eschercia coli	66	70
	Streptococcus pyogenes	52	48
Fungi	*Candida albicans*	81	-

The methanolic extract was ineffective against the fungal strain and ethyl acetate extract exhibited mild activity against fungal strain *C. albicans* at the concentration of 81 mg/ml. Methanolic extract was effective against two of the tested organisms i.e., *S. aureus* and *S.pyogenes*.

DISCUSSION:

Epidemiological evidence implicates bacterial infection as a common triggering stimulus for psoriasis. Recent studies suggest that continuing, subclinical streptococcal and staphylococcal infections might be responsible not only for relapse of acute guttate psoriasis but also for a new episode of chronic plaque psoriasis. Some bacterial antigens appear to have a potential role in the induction of the localized inflammatory response that leads the clinical lesions of psoriasis. These ``super-antigens'' by pass the most restrictive features of antigenic T-cell

activation and stimulate a large proportion of the T-cell population[12].

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The active principles responsible for such activities are helpful in the developing of drugs for the therapeutic use in human beings. Synthetic drugs may cause various side effects. Hence, drugs developed from plant sources can be used in developing newer drugs with minimal side effects[13] and plant based products have been effectively proven for their utilization as source for antimicrobial compounds.

In the present investigation, the antimicrobial and antifungal activity of the ethyl acetate and methanol extract of *G. rottleriformis* bark was assayed against 4 potentially pathogenic microorganisms *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes* and *Candida albican* at different concentrations of the extract to understand the most effective activity. The methanol extract showed a broad-spectrum antibacterial activity with a zone of inhibition of 11 to 18 mm against *Staphylococcus aureus* and 7-14 mm against *Streptococcus pyogenes.* For methanol extract, the maximum zone of inhibition obtained for *Staphylococcus aureus* was 15 mm and for *Streptococcus pyogenes* was 14 mm. All the tested microorganisms exhibited good sensitivity against above three concentrations except *Candida albicans*.

CONCLUSION:

The psoriasis patients are often found to be harboring pathogenic microorganisms and that their Psoriasis improves when they are treated with antimicrobials. Thus, antimicrobial treatment should precede any plan to treat psoriasis patients with anything more than the simplest topical agents. In the present study, methanol extract of *G. rottleriformis* bark exhibited better antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* as compared to the ethyl acetate extract. Among the organisms tested *Staphylococcus aureus* was more susceptible to the ethyl acetate and methanol extract. In conclusion, the bark of the plant *Givotia rottleriformis* is most effective against the tested bacterial strains than the fungal strains and thus the present study supports the early claims of the plant in the treatment of skin diseases like psoriasis.

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