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In vitro antiacne and antidandruff activity of extracted stigmasterol from seed waste of safflower (*Carthamus tinctorius* L.)

Monika Choudhary, Vartika Verma & Nidhi Srivastava*

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan 304 022, India

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Correspondence
Nidhi Srivastava
nidhiscientist@gmail.com

Abstract

The present study deals with the extraction of saponins and their characterization from the seed waste of safflower. The presence of stigmasterol in the extracted safflower seed waste (SSW) was confirmed by Thin Layer Chromatography (TLC), followed by Fourier-Transform Infrared Spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC) on the basis of its peak compared with stigmasterol standard. FTIR showed the identical functional groups of butanolic extract of SSW with standard while TLC and HPLC showed their notable peak and retention time with the same. Further *in-vitro* antiacne and antidandruff microbial activity of extracted stigmasterol was confirmed by disc diffusion method. This preliminary study has exhibited anti-acne and anti dandruff potential of Safflower seed waste extract, in future which could be used in therapies and cosmetic applications.

Keywords: Anti-acne; Anti-dandruff activity; Safflower seed waste; stigmasterol

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1. Introduction

The most important secondary metabolites are flavonoids, saponins, alkaloids, phenolics and tannins (1). These phytochemicals are harmful for microbial cells but are considered significant to therapeutic treatments (2).

In plants, saponins are widely distributed and possess a variety of biological activities. Structure of saponins contains two moieties; a sugar and a non-sugar moiety (aglycone known as sapogenins). Saponins have various biological properties including antioxidant, immune stimulant, anti hepatotoxic, antibacterial, anti fungal, anti carcinogenic, antidiarrheal, anti ulcerogenic, anti oxytoxic, anti hypoglycemic, anticytotoxic and antimolluscicidal (3). Plant based saponins provide abundant assets of antimicrobial compounds (4). For example, the strong activity of saponin (triterpene) isolated from the funicles of

Acacia auriculiformis against microbes has been explored. Saponin causes leakage of certain enzymes and proteins from the cell of microbes (5).

Microbes have the ability to cause various skin related diseases or inflammations including dandruff, infections around, nails, acne skin rashes. Phytochemicals with antimicrobial properties present in plant are of great importance in therapeutic treatment (6). There are reports on strong antimicrobial activity of saponins extract of *Acacia* species against different pathogenic organisms (7).

India has the largest producers of safflower followed by China, U.S.A etc. (8). Safflower is highly rich in PUFA (Poly unsaturated fatty acid) mainly linoleic acid, 76-80% and carthamin (color dye). Commercial safflower seeds have thick and white hull while many experimental types seeds have thin, dark and stripped hull. White hulled varieties have 33-44% hull and 55-65% kernel while 36-42% oil content and experimental thin hulled varieties have 55.5-44.5% hull. Hull makes up a large part of seeds but have no reasonable value. Commercially hull contains large amount of fiber that lowers the market value of hulls and whole safflower meal. A byproduct (Seed meal) is a rich protein source of animal food but quality of the meal is very erratic due to various downstream processes. Safflower seed hull are unpalatable for the livestock feed and it constitutes only a small part of roughage. The seed meal contains phenolic glucosides which have cathartic activity and it can be removed through the extraction process (9). Isolated glucosides may further be used for their biological activities.

2. Materials and Methods

2.1 Materials

Safflower seed waste was collected from safflower oil industry, Lokhande, Latur, Maharashtra. Stigmasterol was purchased from Sigma-Aldrich, USA. HPLC grade water, acetonitrile and silica 20*20 aluminum sheets purchased from Merck, India.

2.2 Preparation of extract for phytochemical screening

Safflower seed waste (SSW) was milled into powder by the grinder. Successive extraction of the sterol based compounds from the safflower seed waste was performed via solvents in a range of nonpolar-polar *i.e.* petroleum ether, chloroform, ethyl acetate and ethanol. The selected 50 % aqueous methanol extract was air dried to get concentrated for further analysis of different class of compounds.

2.3 Phytochemical analysis

Phytochemical screening of aqueous methanolic extracts of SSW was conducted for alkaloids, flavonoids, steroids, terpenoids, tannins and saponins (Table 1) (10).

Table 1. List of phytochemicals analyzed in Safflower seed waste extract.

S.No.	Test	Method	Extract
1	Alkaloids	Alkaloids Mayer's test	+
		Wagner's test	+
		Dragendorff's test	-
2	Terpenoids	Liebermann–Burchard test	+
			-
3	Tannin	Braymer's test	+
		FeCl3 test	-
		Lead acetate test	-
4	Saponin	Foam test	+++
5	Flavonoids	NaOH test	+
		Lead acetate test	+
6	Steroids	$CHCl_3$ test	+
7	Carbohydrates	Benedict's test	+
		Molisch test	+
		Fehling's test	+
8	Protein	Ninhydrin test	+
		Nitric acid test	+
		NaOH test	+

2.4 Extraction of sterol rich compounds

50% aqueous methanol extract of SSW was selected for the further analysis of saponins. Further extraction was done by two phase separation method *i.e.* water and ethyl acetate (11). Aqueous phase which was rich in saponins has been collected separately. Further partitioning was carried out butanol and diethyl ether. Finally, the butanolic phase was collected which was rich in compounds dependent on sterol (12).

2.5 Silica gel column chromatography for purification of plant extracts

Silica gel chromatography was used for separation of sterol rich butanolic (SRB). For separation of the extract, elution solvent ranging from of non polar to polar range was used. The eluted fractions have been accumulated over a certain period of time. Petroleum ether gradation and methanol have been used to elute the compounds.

2.6 Characterization of sterol compounds

Characterization of the SRB extract was done by thin layer chromatography (TLC), Fourier Transform Infra-red Spectroscopy (FTIR) and High performance liquid chromatography (HPLC). SRB extract spot was marked over silica sheets along with standard (stigmasterol). The mobile phase ratio was chloroform and methanol (6:1 v/v) to develop the TLC plates. For spot visualization, vanillin sulphuric acid was sprayed on the plates followed by heating in oven for 100°C.

For identification of various functional groups present in the SRB extract FTIR (Bruker Alpha-T spectrometer) analysis was performed (13). In FTIR spectra, few drops of extract were placed over instrument glass through which the laser passes. Spectra were obtained ion range from 400 to 4000 cm^{-1} and their functional groups were identified in accordance with characteristic peaks (14).

HPLC (Shimadzu LC-10A (Japan)) was carried out to separate and identify the

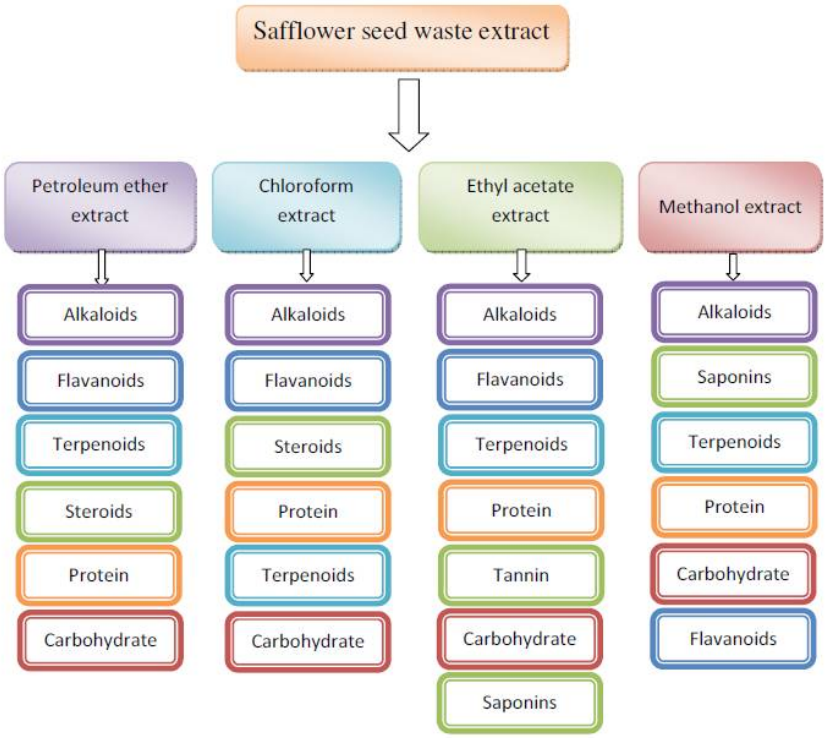

Fig. 1. Phytochemical analysis of different solvent extract of Safflower seed waste.

Fig. 2. TLC fingerprinting profile of Standard Stigma sterol (S) and SRB extract (Sample).

compounds in the SRB extract. 20 µL extract was injected, separated by C-18 Whattman ODS 5 column and detected at 254 nm wavelength. Acetonitrile and water were used as mobile phase with a constant flow rate of 1 ml min^{-1} (15).

2.7 *In vitro* anti-bacterial activity of sterol rich butanolic extract

The antimicrobial efficiency of the SRB extract was performed (16). 50µl of SRB extract was loaded on sterile disc and these discs were placed over already inoculated nutrient plates with acne causing bacteria. These plates were incubated at 37°C for 24 hours. Zone of inhibition was calculated in millimeter. Varying concentration of SRB extract was tested against acne causing microorganism to find the minimum inhibitory concentration (MIC). 0.020 ml of overnight culture of bacteria at 37°C was added in 10 different test tube containing 10 ml of nutrient broth. Varying concentrations of SRB extract was added to each test tube was incubated for 24 hr and absorbance was recorded at 600nm. General antibiotic (streptomycin) was used as a control.

2.8 *In vitro* anti-fungal activity of sterol rich butanolic extract

Specific extract concentrations were prepared in sterilized water and the resulting extracts were used to evaluate their antidandruff activity by means of a disc diffusion method. On Sabouraud's agar supplemented with olive oil, dandruff sample was inoculated and incubated at 37˚C (17). The diameter of inhibition zone according to the concentration of extract was measured in millimeters. Experiments were carried out with triplicates. The procedure was also performed for dandruff causing fungus for the standard antifungal (fucanozol) without SRB extract (standard). A plate with Sabouraud's dextrose agar was prepared as a control.

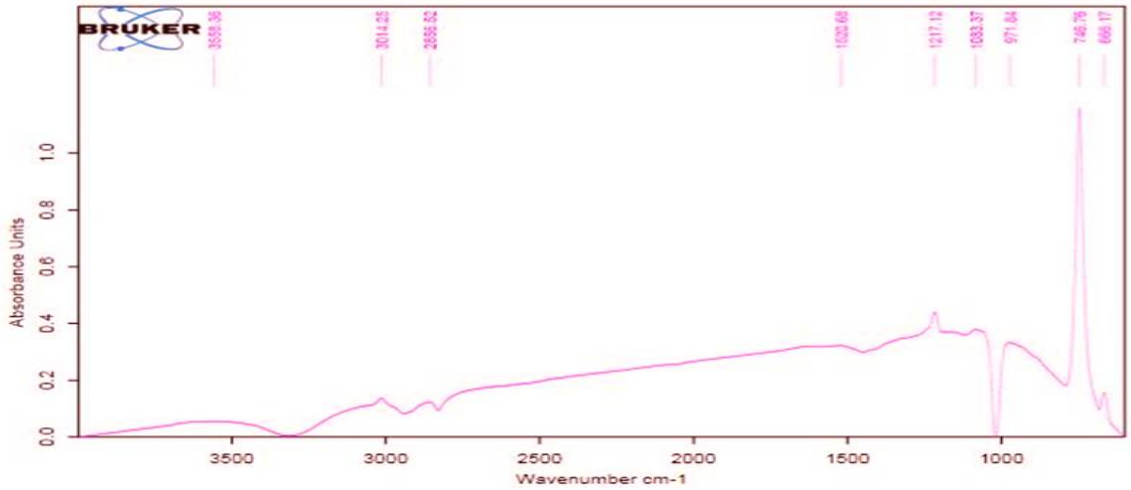

Fig. 3. FTIR peaks of SRB extract.

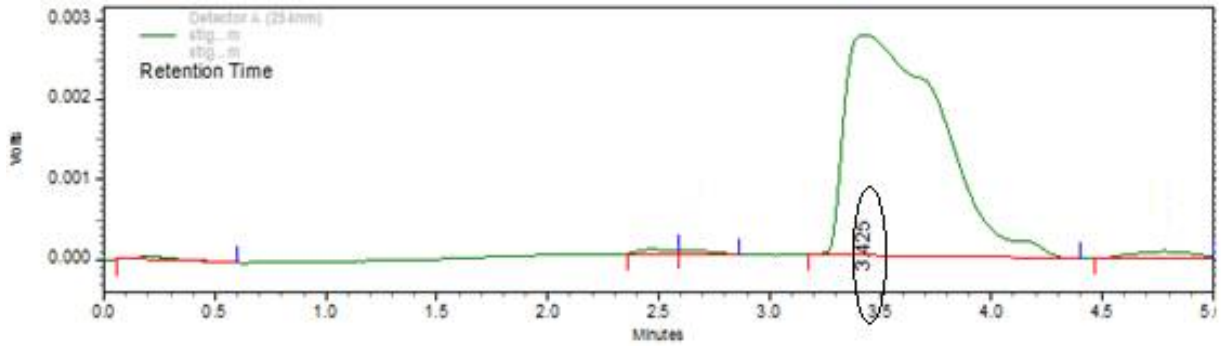

Fig. 4. (a). HPLC chromatogram of standard stigmasterol.

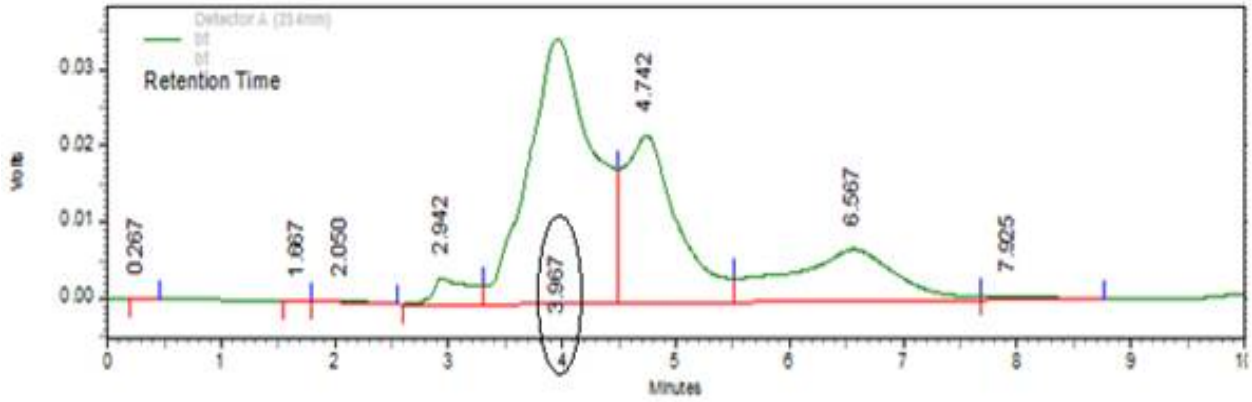

Fig. 4 (b). HPLC chromatogram of SRB extract of safflower seed waste.

3. Results and Discussion

3.1 Phytochemical analysis

The methanolic extract of SSW showed the maximum presence of saponin than ethanol, petroleum ether respectively whereas in chloroform extract it was absent. The foam test gave the confirmation of the presence of saponin (18).

The methanolic extract has shown the presence of various bioactive compounds *viz*. terpenoids, steroids, tannins, saponin, alkaloids, proteins, flavonoids and carbohydrates (Fig. 1).

Similarly, there are reports on the presence of compounds like glycosides, coumarins, polysaccharides, steroids and fatty acids in extracts of SSW (19).

3.2 Characterization of sterol compounds

The thin layer chromatography of both normal and reverse phases provides excellent qualitative data for estimating plant material saponins (20). There were various spots of SRB extract after separation present on TLC, but one spot (RF 0.67) coincided with that of standard stigmasterol (R_f 0.665). The spots turned violet when vanillin spray was sprayed. The values for these spots were calculated as for SRB extract and for the standard (Fig. 2).

The FTIR analysis of butanolic extract of leaf showed distinguished and characteristic peaks at 3558.35, 3014.25, 2856.62, 1620.69, 1217.12, 1033.37, 971.84, 746.76, 666.17 corresponding to alcohols, alkanes, alkanes, alkanes, alkanes, alkenes, alkenes, alkenes, alkenes respectively (Fig. 3).

According to previous findings, similar results were obtained in aqueous methanol extract of sea grass (21).

HPLC on C_{18} reverse-phase column have been used for separation and identification of steroidal saponins of *Digitalis purpurea, Convallaria majalis* and *Asparagus officinalis.* Acetonitrile and methanol were used for gradient elution (22). HPLC quantification of stigmasterol as a standard, showed retention time at 3.425 min (Fig. 4a) while SRB extract showed retention time at 3.967 min (Fig. 4b).

The obtained peaks were the nearly same due to the presence of different sterol rich compounds. Previous studies on *Bupleurum falcatum* showed the presence of saikosaponin a, c and d compounds (23).

3.3 *In vitro* antiacne and anti dandruff activity

Antibiotic activity testing of SRB extract was conducted using disc diffusion against acne-causing bacteria. The zone of inhibition was measured and diameter of 11 mm for SRB extract, 20 mm for antibiotic was recorded whereas there was no inhibition of microbial growth observed in control (solvent) (Fig. 5).

Hence SRB extract showed significant antimicrobial activity against acne causing bacteria. The methanolic extract of *Curcuma longa*

rhizome was tested against *Staphylococcus aureus* which showed satisfactory antimicrobial activity (24).

In the present study, the zone of inhibition recorded was 11 mm for isolated SRB and no visible zone was observed against butanol control, hence it is concluded that SRB extract is potent against acne causing bacteria.

Antidandruff activity of SRB extract was also checked by disk diffusion method. The zone of inhibition against dandruff causing microbes was measured 9 mm for SRB extract, 3 mm for Antifungal (fucanozol), and there was no zone of inhibition of solvent (control) (Fig. 6).

Table 2. MIC values of the diluted SRB extract.

Dilution of SRB Extract	MIC of SRB extract
Standard	0.599
2X	0.439
4X	0.387
6X	0.280
8X	0.256

These results confirmed the antidandruff activity of SRB extract. Similar results were obtained from ethanolic extract of *Hibiscus rosasinensis* against dandruff causing *Malassezia furfur* (25).

Fig. 5. Anti acne activity of SRB extracts (SR), Solvent (S), and Antibiotic streptomycin (Ab).

Antimicrobial compounds isolated from plants act as a good source of medicine because of their large therapical ability (26). Roots of *Cleome ciliata* Schum. & Thonn. have a variety of bioactive compounds like terpenoids, alkaloids, tannins and saponins that posses antibacterial and antifungal properties (27). Table 2 revealed minimal inhibitory concentration (MIC) values of acne-present micro organisms.

The highest MIC value was in 2 time's diluted extract. The lowest MIC value was found in 8 time diluted extract.

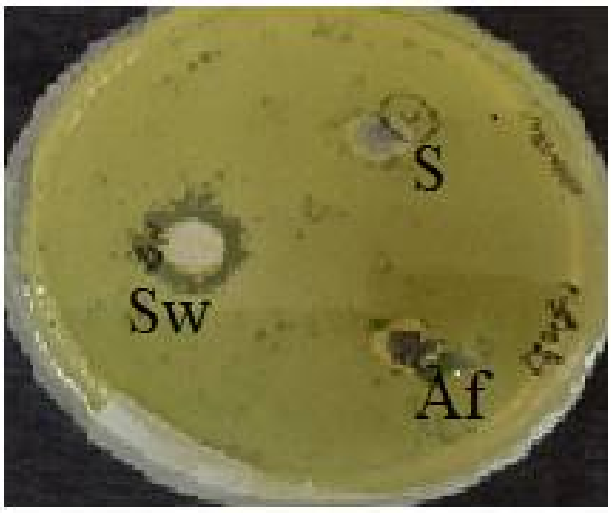

Fig. 6. Anti dandruff activity of SRB extract (Sw), solvent (S), Antifungal fucanazol (Af).

Significant results have been obtained against the dandruff in the petroleum ether extract of *Tridax procumbens* (28). The green tea seed extract is a high amount of saponins which showed antibacterial actions against *Streptococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 25922), and six salmonella strains (29). The form forming ability of saponin also help to destruct the cell wall of microbial cell that further cause the leakage of proteins and enzymes from that cell. The antibacterial activity of *Cassia auriculata* extract is possibly linked to the presence of flavonoids, steroid and saponins (30).

4. Conclusion

Though safflower is oil yielding rabi crop and highly rich in secondary metabolite. Its seed coat has been found rich in some antinutritional factor (saponin etc). Therefore, the current study follows to isolate and characterize the saponin compounds from the seed waste, indirectly participating in the environmental waste management. Since saponin acts to control the wide range of microbial growth, so expected saponin were tested against acne and dandruff causing microbes. In which stigma sterol has been given better response to control the growth of these microbes. The aim of the study was reutilization of these natural compounds present in waste for their maximum participation in pharma industry. In future our findings with more novel compounds present in safflower seed waste with detailed mechanism of action against acne and dandruff causing microbes may add a new step in pharma industry.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contribution

MC has performed the experiment under the guidance of NS; NS has designed the entire

concept, analyzed the data, edited and finalized the manuscript. VV has helped in the experimental procedures along with data compilation.

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