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Investigation on Eco-Friendly Natural Dyes: Chemistry, Dyeing and Antimicrobial Properties of *Helichrysum bracteatum*

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The present textile processing methods causes enormous damage to environment by the way of water pollution by releasing toxic chemicals, non-biodegradable dyes, total dissolved solids and sludge from effluent treatment plants. Thus the need of the hour is to find eco-friendly methods of dyeing. One such possibility of eco-friendly dyeing is to screen natural resources for the industrial dyeing application. The present work involves to investigate a new plant sources for dyeing textile materials. Accordingly the *Helichrysum bracteatum* herb with golden yellow flowers was investigated. It was found to contain major two flavonoids namely 6,3',4',5'-tetrahydroxy-4-O-β-D-glucopyranosylaurone (bractein) and 6,3',4'-trihydroxy-4-O-β-D- glucopyranosylaurone (cernuoside). Further on dyeing with natural and metallic mordants various intensities of colour shades for fabrics with acceptable fastness properties was obtained. It is worthy to note that no toxic chemicals were utilized in the entire process. On testing for antimicrobial activity, samples showed remarkable bacterial activity in assay conditions on *Escherichia coli*, *Proteus vulgaris* and *Salmonella typhi*. With increasing concentrations of dye, the zone of inhibition is increasing almost linearly.

Key Words: *Helichrysum bracteatum*, Extraction, Polyphenolics, Dyeing, Antimicrobial activity.

INTRODUCTION

The harmful effects of synthetic dyes on environment and human beings made worldwide need to develop the eco-friendly and safe dyes that may be used for textile and other applications including food and pharmaceuticals[1]. To start with, German has banned certain synthetic azo dyes, which are allergic and carcinogenic. Thus emphasis on the screening of newer natural resources of natural material for the industrial application has gained momentum. Further the present textile processing methods causes enormous damage to environment through water pollution by releasing toxic chemicals, non-biodegradable dyes, total dissolved solids and sludge from effluent treatment plants.

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Vegetable dyes have been used for thousands of years[2]. With the present awareness on various harmful effects of synthetic dyes, the ideal choice is natural dyes. In spite of a few drawbacks, the world requirement of natural dyes is growing at a faster rate. The present status of the natural dyes was critically discussed[3].

In the light of knowledge gained in recent times on the chemistry of colours and various factors responsible for fastness characteristic of these colours on textiles, it is necessary to carry out a comprehensive survey of promising flora containing the dyes, extraction methods and its application. To make success in this venture, good depth of knowledge in plant taxonomy, organic natural products chemistry and textile chemical processing is necessary. Only the need of the hour is to find the various new sources of natural dyes and application as value added product with the scientific approach. Thus there is a great chance that natural dyes can act as therapeutic agents[4].

The authors group has been concentrating research work on the investigations on new natural sources for textile dyes available in the hilly areas of south India, which are hither to be investigated. Further the interest will be on the extraction methods of colouring matter, purification and structural elucidations and biological activity of the active ingredients. In this paper the availability, extraction, characterization and dyeing of natural colouring material from the flowers of *Helichrysum bracteatum* has been described. As a value added application, the material was screened for antimicrobial activity.

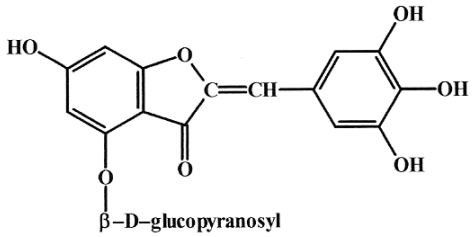
EXPERIMENTAL

Helichrysum bracteatum, belongs to the family of Asteraceae, is stout annual herb, naturalized in high altitudes. It is commonly available in the Nilgiri and Kodaikanal hills of south India[5]. The flowers are large, everlasting and available in golden yellow, pink and ivory white shades. *Helichrysum* species are rich in secondary metabolites and have good medicinal value[6]. Previous reports reveals that *H. bracteatum* contains lignin and aurones[7]. For determination of antimicrobial activity the dyes isolated were tested with and without mordant in solution. *Escherichia coli, Proteus vulgaris* and *Salmonella typhi* of microorganisms were procured from Department of Biotechnology, Kurinji College of Arts and Science, Trichy, India and used for the study.

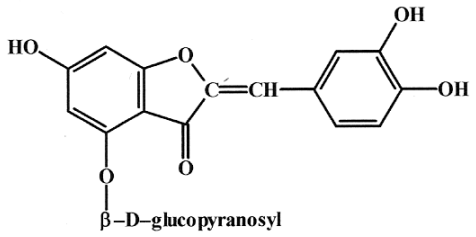
Equipment: For dyeing, a julabo SW22 shaker water bath with automatic temperature control was used. For the determination of the antimicrobial activity, glassware and culture media were sterilized in an autoclave at 120 ºC for 0.5 h. An incubator of both static and rotary shaker type (Orbitek Ltd) was used for incubation and growth of the bacteria. Optical growth was measured using Specord 200 spectrophotometer. Inoculation was carried out in a vertical chamber equipped with laminar air flow, an ultraviolet lamp and a burner (Atlantic).

Extraction and purification of dyes: Fresh golden yellow flowers of *H. bracteatum* were collected from the Nilgiri hills of south India during June 2007.

The flowers were shade dried and then dissolved in distilled water under boiling condition for 0.5 h. The colouring matter was extracted, filtered and preserved. The filtrate is used for dyeing after standardization and the residue, which is mainly the agro waste, is utilized for composting manure. The filtrate was subjected to various testing, including analysis by TLC and UV Visible spectrophotometer. On development of TLC on cellulose, using 50 % acetic acid, five UV active compounds were observed, out of which major two are bright yellow. All the reactions were indicative of polyphenolics. The yellow active compound showed positive reaction for aurones and characterized as bractein (6,3',4',5'-tetrahydroxy-4-O-β-D-glucopyranosyl-aurone) and cernuoside (6,3',4'-trihydroxy-4-O-β-D-glucopyranosylaurone), which was further confirmed by employing various physical, chemical and spectroscopic data (m.p., UV, IR, ^{1}H NMR, FDMS, hydrolysis and co-spot)[7], whose structures are given in Fig. 1.



6,3',4',5'-Tetrahydroxy-4-O-β-D-glucopyranosylaurone (Bractein)



6,3',4'-Trihydroxy-4-O-β-D-glucopyranosylaurone (Cernuoside)

Fig. 1. Structure of colourants

Mordanting: Natural dyes may require some mordanting agents in order to produce affinity between the fibre and the dyes. In present experiments, the natural mordant, tannic acid has been used to attain good fastness and different shades. A few eco-friendly metallic mordants such as stannous chloride, alum, ferrous sulphate and calcium hydroxide are also employed in minimum concentration. Accurately weighed cotton samples were treated with tannic acid [4, 8 and 10 % owf] at room temperature for *ca.* 4 h (MLR 1:25). After the completion of mordanting under the constant stirring for *ca.* 4 h, the samples were dissolved in metallic salt (MLR 1:20) for *ca.* 2 h at room temperature.

Dyeing: The shade dried flowers in distilled water (1:10) are allowed to boil for *ca.* 0.5 h. The hot dye solution is filtered in order to avoid the unwanted dust particles that are hindrance to subsequent dyeing procedure. The filtered solution is boiled again to get a concentrated dye solution suitable for dyeing the textile materials. Dyeing has been tried in cotton by the following process: The dye bath is prepared with the extracted dye solution and then mordanted material is entered into the dye bath at a room temperature with constant stirring. The material is allowed to be at the dye bath for *ca.* 2 h at 60 ºC.

Washing: After dyeing, the material is squeezed and washed in running cold water followed by hot water at 80 ºC and then dried to obtain the coloured fabric. The details of colour retention and light fastness properties of the fabric material are given in Table-1.

TABLE-1
FASTNESS PROPERTIES OF DYE EXTRACT OF
H. bracteatum ON COTTON MATERIAL

Mordant	Mordanting method	Dyed shade	Fastness properties			
			Wash (40 ºC)	Light	Rubbing	
					Dry	Wet
Stannous chloride	Pre	Pale yellow	3-4	4	5	5
Alum	Pre	Pale grey	3-4	4/5	3	3
Ferrous sulphate	Pre	Dark grey	4/4	4	5	5
Calcium hydroxide	Pre	Yellowish orange	4/5	4	4	4

Fastness properties: The wash fastness tests of dyed samples were determined by the method described in BS EN 20105-C 02:1993 and the light fastness by the method described in BS EN 20105-B 02:1993[8]. The dry and wet-rub fastness tests of the dyed samples were determined using the crock tester.

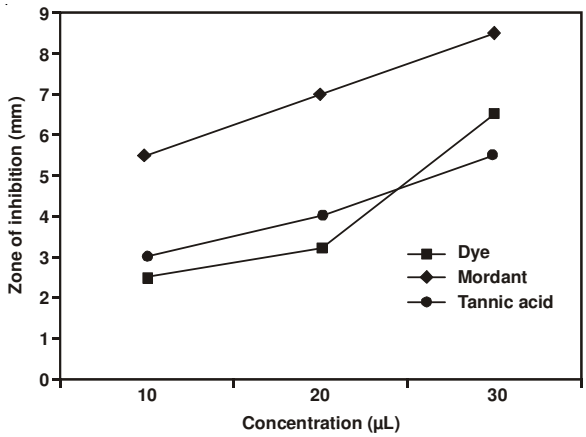
Antimicrobial activity: Test organism cultures of *E. coli, P. vulgaris* and *S. typhi* were used in the study. Nutrient agar medium (g/L: peptone 5.0, beef extract 1.5, yeast extract 1.5, NaCl 5.0, agar 20, pH 7.5) was prepared and autoclaved at 121 ºC for 20 min. Sterilized petri plates were prepared with an equal thickness of nutrient agar. Test organisms were grown overnight at 37 ºC, 120 rpm in 10 mL nutrient broth. The dye obtained from the flowers of *H. bracteatum*, natural mordant tannic acid and metallic mordant were studied against microorganisms. This broth was used for seeding the agar plates. 10, 20 and 30 µL of 10 % dye, 2 %, tannic acid and 4 % stannous chloride were impregnated onto a small disc of filter paper (diameter 5 mm) and placed on top of the seeded medium. After overnight incubation at 37 ºC the zones of inhibition were measured. The average width of the zone of inhibition on either side of the dye discs were calculated using following equation:

$$W = (T-D)/2$$

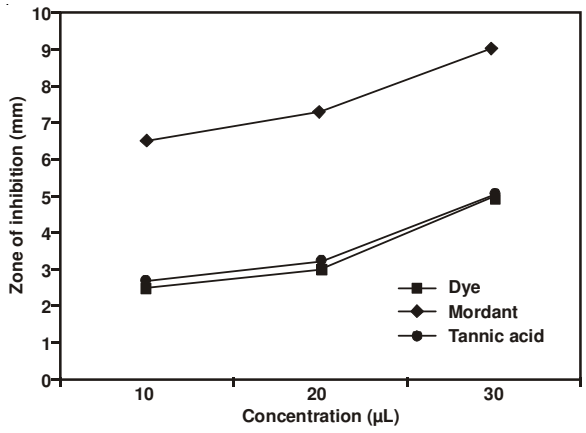
where W is the width of the zone of inhibition (mm), T is the total diameter of the test specimen and clear zone(mm) and D is the diameter of the test specimen itself (mm).

Preliminary evaluation of antimicrobial activity through the agar diffusion method: The diffusion method was used as a preliminary test for detecting antimicrobial activity in substances[9] *i.e.* the dye obtained from the flowers of *H. bracteatum*, natural mordant tannic acid and metallic mordant stannous chloride. Since the diffusion phenomenon depends on each substance's physico-chemical properties, for example in diffusion co-efficient as well as the medium where the diffusion occurs, it is possible to obtain a qualitative indication of antimicrobial activity.

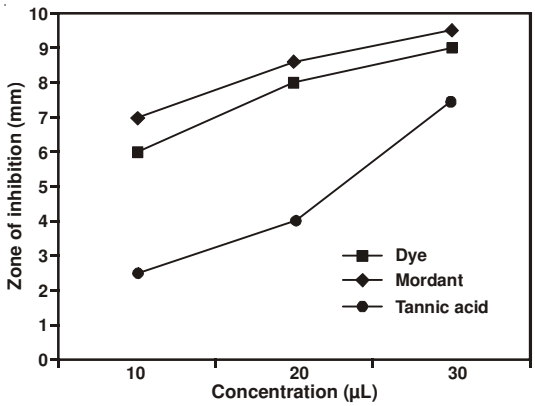
Therefore, the inhibition zone diameter measurements cannot be considered to be a comparative parameter when different formulations are tested, nor can the absence of antimicrobial activity be assured when the inhibition zone is not formed. The assays showed antimicrobial activity in all tested samples with different inhibition zone (Fig. 2).



Effect of dye, mordant and tannic acid against *E. coli*



Effect of dye, mordant and tannic acid against *P. vulgaris*



Effect of dye, mordant and tannic acid against *S. typhi*

Fig. 2. Antibacterial properties of dye, natural and metallic mordant

RESULTS AND DISCUSSION

The plant extract was subjected to TLC analysis after a preliminary purification. The major compounds were characterized as 6,3',4',5'-tetrahydroxy-4-O-β-D-glucopyranosylaurone (bractein) and 6,3',4'-trihydroxy-4-O-β-D-glucopyranosylaurone (cernuoside). These flavonoids were known to be form stable colours in dyeing[10]. In all the samples mordanting was carried out. Stannous chloride gave pale yellow and alum gave pale grey. With ferrous sulphate and calcium hydroxide, it yielded dark grey and yellowish orange respectively. The increase in the concentration gave deep shades. The best results were observed for 4 % mordant owf in terms of shade. The results of stable different shades are represented by PANTONE Textile Colour Swatch Card Numbers *i.e.* pale yellow (13-0932 TC), pale grey (13-1016 TC), dark grey (17-1502 TC) and yellowish orange (13-0935 TC)[11]. The wash, light and rubbing fastness are given in Table-1, which is indicative of moderate fastness.

Antimicrobial activity was carried out for different concentration of dye, natural mordant and metallic mordant with different concentration (10, 20 and 30 µL) against the microorganisms *E. coli*, *P. vulgaris* and *S. typhi*. The zone of inhibition was recorded in each case (Fig. 2). It was observed that the increase in concentration (30 µL) of each of dye, natural mordant and metallic mordant leads to increase in inhibition, as reflected by enhancement in diameter.

The natural mordant (tannic acid) with 2 % concentration of 10, 20 and 30 µL exhibited clear zone of inhibition when we use the microorganism *S. typhi* with 30 µL. In case of dye [10 % concentration of 10, 20 and 30 µL] showed the clear zone of inhibition when we use the microorganism *S. typhi* with 30 µL. The microorganism *E. coli* with 30 µL concentration also exhibited considerable amount of inhibition zone. The metallic mordant stannous chloride [with 4 % concentration of 10, 20 and 30 µL] the microorganism *S. typhi* and *E. coli* exhibited highly inhibition zone.

The microorganism *P. vulgaris* with 30 µL also exhibited considerable amount of inhibition zone. It may be concluded that in all the cases the microorganisms *E. coli* and *S. typhi* are highly effective antimicrobial agents. Dye and mordants showed highest activity against *E. coil* and *S. typhi* at highest test concentration (30 µL). In all the cases the microorganism *P. vulgaris* showed a small zone of inhibition except in the case of metallic mordant stannous chloride, where it showed maximum zone with test concentration 30 µL.

Conclusion

The present experiment involves in the investigation of new plant sources for dyeing textile materials. It is well known that the flavonoids form a stable colourants in dyeing. Accordingly the *H. bracteatum* contains good quantity of flavonoids namely bractein and cernuoside. In this experiment an economical method of aqueous dye extraction was attempted successfully. Further various intensities of colour shades for fabrics were obtained using known quantity of dye solution and various mordants. The common fastness properties obtained indicated that the dye from *H. bracteatum* will serve as good natural dye. The plant species can be self propagated. It is worthy to note that no toxic chemicals were utilized in the entire process. Thus the dyeing method will not cause any noticeable pollution. The tested samples showed remarkable bactericide activity in assay conditions on *E. coli*, *P. vulgaris* and *S. typhi*. With increasing concentrations of dye, the zone of inhibition is increasing almost linearly and thus it is apparent that the selected dyes are bacteriostatic.

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