Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.8, 2015

Evaluation of Antibacterial Activity of Flavonoid and Oil Extracts from Safflower(Carthamus tinctorius L)

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Abstract
Flavonoid and oil extracts were extracted and tested for their *in vitro* antibacterial activity against two different pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* , the results reflect promising moderate to good activity at different dilutions against different strains of bacteria employed.
Keywords: Safflower , Flavones, oil extract, Antibacterial activity.

INTRODACTION

The use of plant based medicines (local medicine) date back to (4000-5000) B.C. Furthermore according to WHO about 80% of world population depend on medicinal plant for their health care needs, and more than 30% of pharmaceutical preparations are based on plants(1,2).

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, *i.e*. any part of the plant may contain active components(3).

Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances proteins, amino acids, bioactive peptides, phyto hormones, sugars, flavonoids, and bio pesticides (2,4).

Safflower(*Carthamus tinctorius* L), a member of Asteraceae family, is a famous traditional Chinese medicine which has many effects such as anticoagulant, vasodilator, antioxidant, immunosuppressant, and neuro protector [5]. Safflower (*Carthamus tinctorius* L.) flowers produce red and yellow pigments. Red safflower pigment was originally used as a cosmetic and textile dye, and today is also used as a food colorant. The main component of the red pigment is water, the red pigment is mainly used in colored chocolate (6).

So in the present investigation, we report herein the extraction of flavonoid and oil from *Carthamus tinctorius* with an aim to find most active antibacterial agents of these extract with different concentrations.

All the compounds were tested for their *in vitro* antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Materials and Methods

Plant Material & Chemicals:

The (Safflower (*Carthamus tinctorius* L.)) plant used in this study was obtained from the local markets . All of the chemicals were purchased from Sigma- Aldrich Co. (St. Louis, MO,USA), and the solvents were obtained from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

Preparation of extracts

Petroleum ether Extract:

25.000 gm of safflower flowers powder was added to a thimble and then placed in a Soxhlet extractor . Heat was applied to a round bottom flask which contain petroleum ether solvent was placed at the base of the Soxhlet extractor. The extract was concentrated using a rotary evaporator (PuchiRotavapor-Re) then dried at room temperature (7), the weight of brown oil was 4.459 gm.

Flavonoid extract:

25.000 g of safflower flowers powder soaked in 300ml (70% ethanol) for 6 hours, the extracted filtered through filter paper (whatman No.541), 2% aqueous lead acetate then added until the formation of flocculent and brown precipitate , the precipitate separated by filter paper(whatman No.532) and washed with water, methanol and ethyl acetate consecutively.

The salt that produced converted to chloride by dissolving in (50ml acetone and 10ml 2N HCl) and filtered through filter paper (whatman No. 540) .The filterate placed in Petri dish at room temperature until it dry .The weight of amorphous red powder that formed was 5.067 gm.

Preliminary qualitative test:

Preliminary tests were carried out on the petroleum ether extract and flavonoid extract as showed in table (1).

Thin layer chromatography
(TLC) were carried out on the Petroleum ether extract (benzene : Chloroform (1:1)) and Flavonoid extract by using (Sec-butanol :formic acid : water (7.7:1:1.3)).

Antibacterial Activity
Cultures
The Cultures of the bacteria used Escherichia coli and Staphylococcus aureus were procured from Department of Biology. Cultures were maintained on the medium suggested by the respective laboratory and sub-culturing was done fortnightly.

Antibacterial Activity of Flavones and oil extract
The experiments were performed to check the antibacterial properties of flavonoid and oil extracts against selected bacterial pathogens. The compounds were dissolved in Dimethyl Sulphoxide DMSO in different concentration and accordingly these dilutions were made the activity was done by Well method(8). The zone of inhibitions in mm recorded for different dilutions of flavonoid and oil extract against the bacteria are tabulated as Table 3.

Results and Discussion
The flavonoid and petroleum ether extracts were isolated in an yield 20.268% and 17.836% respectively from the dried petals.

Table (1) indicate the preliminary phytochemical analysis for flavonoid extract and petroleum ether extract. The results revealed that there was no alkaloids, carbohydrate , glycosides, saponins and tannin in petroleum ether but contain phenols ,steroid and terpinoid. The flavonoid extract contained only flavonoid compound. TLC procedure was run for this compounds and the results were shown in table (2).

The search for new natural antioxidant and antimicrobial agents from plants has been interested. In a search for new plant products derived photochemical or biological active compounds against chronic and degenerative diseases (9).

Modern pharmacological experiments have demonstrated that safflower with its active compounds possesses wide-reaching biological activities, including dilating coronary artery, improving myocardial ischemia, modulating immune system, anticoagulation and anti thrombosis, anti oxidation, anti-aging, anti hypoxia, anti fatigue, anti inflammation, anti hepatic fibrosis, antitumor, analgesia, etc.(10)

More than 200 compounds have been isolated from *C. tinctorius* and the commonly known ones are flavonoids, phenylthanoid ,glycosides, coumarins, fatty acids, steroids and polysaccharides. Important chemical compounds present in safflower. Oil content of the seeds is similar to that of olive and includes linoleic acid, oleic acid and linolenic acid, Luteolin and its glucopyranosides have been also found in the leaves. A new quinoc - halcone C-glycosides, tinctormine, was isolated from the plant together with safflower yellow *B. Nicotiflorin* is a natural flavonoid extracted from coronal of *C. tinctorius* (11).

Flavones constitute one of the major class of naturally occurring compounds. Synthesis and antibacterial, antifungal activity studies of Flavones and their derivatives have attracted considerable attention due to their significant biological activities (12-14).

Greater and remarkable antibacterial activities of the flavones and oil tested extracts were recorded with *Staphylococcus aureus* and *E. coli* (table 3). These extracts showed better activity against the Gram-positive than the Gram-negative bacteria. The results support the observation that Gram-negative bacteria are more resistant, probably because of their thick murein layer which prevents the entry of inhibitors, due to that the cell membrane of gram negative bacteria consisted of many condensed fat bi layers lipid compared with gram positive cell membrane (15).

It was clear that, different concentrations of the flavonoids 25 µg/ml, 75µg/ml, 250µg/ml, 500µg/ml, 1000 µg/ml and 100000µg/ml tested in the experiment were much effective compare to the same concentrations of oil extract, the flavones tested produced varied inhibition zones.

The flavones with more number of –OH groups inhibited both Gram-positive bacteria, and the Gram-negative, thereby suggesting a broad spectrum antibacterial.

Table (1): Preliminary qualitative test for petroleum ether and flavonoid extract

Phytochemical	Petroleum ether extract	Flavonoid extract
Glycoside	-	+
Phenols	+	+
Flavonoids	-	+
Tannins	-	-
Saponins	-	-
Alkaloids	-	-
Terpenoids	+	-
Sterols	+	-
Carbohydrate	-	-

Absence = - ; Presence = +

Table (2): TLC for preliminary qualitative test for petroleum ether and flavonoid extract

Test sample	P-ansaldehyde & Phosphoric acid	Ninhydrin	Folin reagent	Drangdroff	40% H_2SO_4	H_2SO_4 + Chloroform	visible
Petroleum ether extract	**0.92** **0.68** 0.569 0.38 0.253 0.126 0.06	-	-	-	**0.92** **0.68** 0.569 0.38 0.253 0.126 0.06	**0.92** **0.68** 0.569 0.38 0.253 0.126 0.06	-
Flavonoid extract	-	-	0.36 0.22 0.15 0.07	-	0.36 0.22 0.15 0.07	-	0.36 0.22 0.15 0.07

Table (3.): Antibacterial activity of flavonoid and oil extracts against pathogenic (G+) and (G-) bacterial strains.

Bacteria / Extracts and conc.		*S. aureus* (Pathogenic)	*E. coli* (Pathogenic)
Flavonoid	25	10	11
	75	19	9
	250	24	20
	500	30	18
	1000	32	30
	100000	29	20
Oil	25	11	9
	75	10	8
	250	30	13
	500	38	20
	1000	39	29
	100000	19	11

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