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Phytochemical Screening and Biological Activities of the Camel Thorn *(Alhagi kirghisorum)* and Safflower Flowers *(Carthamus tinctorius* L.) Grown in Kazakhstan

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Abstract: *Alhagi kirghisorum* and *Carthamus tinctorius* L are two important kinds plants of Kazakhstan. In the present study attention is being paid to medicaments comprising antibiotics composed of extract obtained from medicinal vegetative raw materials (*Alhagi kirghisorum*) method of supercritical carbon dioxide extraction. The second vegetative raw materials *Carthamus tinctorius* L. is commonly known as Safflower. *C. tinctorius* extracts and oil are important in drug development with numerous pharmacological activities in the world. Ethyl acetate and methanol extracts of the *Carthamus tinctorius* L., for treatment of skin infections were evaluated in-vitro for antimicrobial and antimalarial properties. Antimicrobial, antimalarial and anti-leishmanial assays were analysed for the extraction and its major components. Preliminary phytochemical analysis of the extracts indicated the presence of steroids, glycosides, saponins and tannins.

Key words: *Alhagi Carthamus tinctorius* · Asteraceae · Safflower · Phytochemistry · Biological activity

INTRODUCTION

The genus *Alhagi* is in the family Leguminosae in the major group Angiosperms (Flowering plants). The Plant List includes 21 scientific plant names of species rank for the genus *Alhagi*. Of these 9 are accepted species names. It is a plant of tropical and subtropical regions, found in Africa, Asia, US, Europe and Middle East. Common in disturbed Urban sites, abundant along riverbanks, canals, irrigation ditches and sometimes in cultivated field. Common along Arabian Gulf Coast; less frequent inland. [l-3]. Hepatotoxicity implies chemical-driven liver damage. Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometime even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins [4]. Biochemical markers (i.e. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage. Liver damage is further characterized into hepatocellular (predominantly initial Alanine transferase elevation) and cholestatic type (initial alkaline phosphatase rise). However, they are not mutually exclusive and mixed type of injuries is are often encountered. Chemicals and drugs such as Carbon tetrachloride (CCl4) and acetaminophen catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes in to the blood [5]. Flavonoids and other phenolics of plant origin have been reported to have roles as scavengers and inhibitors of lipid peroxidation [6-8].

Carthamus tinctorius (L.) It belongs to Asteraceae family in the order of Asterales that contains about 22,750 genera and more than 1,620 species. Carthamus species probably originate from Southern Asia and is known to have been cultivated in China, India, Iran and Egypt almost from prehistoric times. During middle ages it was cultivated in Italy, France and Spain and was introduced into United States in 1925 from the Mediterranean region. *C. tinctorius* has been known as "Golrang" in Iran. It is grown for the red/orange pigment in the flower petals which is used for coloring rice and bread and for dyeing

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cloth. After synthetic aniline dyes took over this market in the 1800's the crop was grown as an oilseed [9]. The genus *Carthamus* from the Asteraceae family comprises 16 recognized species [10]. *C. tinctorius* is the only cultivated species of this genus, but the others are either wild or weeds. *Carthamus oxyacantha* as one of the wild species is widespread in Turkey, subtropical regions of western Iraq, Iran, Northwest India, throughout Kazakhstan, Turkmenistan and Uzbekistan. It contains a high amount of polyunsaturated fatty acid linoleic acid (70%) and monounsaturated oleic acid (10%) with small amounts of stearic acid [11]. The flowers of *C. tinctorius* are an important medicinal material in prescriptions used for cardiovascular, cerebrovascular and gynecological diseases. In China, the water extract of *C. tinctorius* has been developed as an intravenous injection, which is extensively applied to treat cardiovascular diseases clinically [12]. Its dye is mainly used as a coloring agent [13].

MATERIALS AND METHODS

Chemical Constituents: Plant *Alhagi kirghisorum* have alkaloids, flavonoids, glycosides, steroids, terpenoids, resins and tannins are found in different extracts. Quantitative analyses of important inorganic elements have been performed in ash [14].

Laboratory organization translational medicine in Kazakh National Medical University as shared laboratories. The Institute established the Center Atchabarova pharmaceutical development (Research and Development) which will implement a comprehensive pharmaceutical development of a full cycle: synthesis and screening of new molecules, development, analysis and implementation of new drugs and medical products (diagnostic systems) into production. Also pharmaceutical development of innovative medicines.

Plant Materials: The plant materials used in study were obtained from southern Kazakhstan, safflower (*Carthamus tinctorius* L.) collected in the summer 2013. The plant was identified by taxonomist Konyrbekov M. of the station. A voucher specimen was deposited at the herbarium Krasnovodopadskaya Breeding Experimental Station, Ministry of Agriculture, Republic of Kazakhstan.

Extraction: The whole plants (*Carthamus tinctorius* L.) were air-dried at ambient temperature over a period of 6 days. For isolation of biologically active compounds carried out the selection of solvents, optimized process conditions. In order to optimize the extraction of biologically active substances, the influence relations, raw materials, solvent, extraction time and temperature. The most appropriate of 70% methyl spirit (at a ratio of raw material: extractant 1:6-8, 3 days, room temperature) in these conditions are extracted up to 60% of biologically active substances. The total weight of the plant was 1 kg and from this 15 g of hexane, 50 g ethyl acetate and 35 g of methanol extracts was obtained by extraction. The extracts were stored in universal bottle and refrigerated at 4°C prior to use.

Chemical Composition: More than 200 compounds have been isolated from *C. tinctorius* and the commonly known ones are flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids and polysaccharides [12]. Analysis of safflower seeds showed that crude protein ranged from 14.9% to 17%, total sugar from 3.2% to 9.2% and extractable lipids from 25% to 40% [15]. Oil content of the seeds is similar to that of olive and includes linoleic acid (63%-72%), oleic acid (16%-25%) and linolenic acid (1%-6%) [16].

Phytochemical Study: Crushed air-dry raw material (1300 g) subjected to extraction by the infusion of 70% aqueous methanol at room temperature for 3 days. Extraction was repeated twice. The combined extract was concentrated and successively extracted with petroleum ether, ethyl acetate, with the result obtained by 3 workers extract - petroleum-ether, ethyl acetate, methanol. Preliminary phytochemical screening of *Carthamus tinctorius* L, showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. The *Carthamus tinctorius* L. religiosa showed the presence of, lanosterol, β-sitosterol, stigmasterol. The methods of two-dimensional paper chromatography (PC) and thin-layer chromatography (TLC) in various solvent systems using specific developers found that the major groups of biologically active substances of plant aerial part are investigated saponins, flavonoids, amino acids, mono-, oligo- and polysaccharides, phenolic acids.

Phytochemical Screening: The plant extracts were screened for alkaloid, steroid, saponin, reducing sugar and other glycosides using the standard laboratory procedures [17].

Biological Activity

Antimicrobial Assay: The oil and isolated compounds from *Carthamus tinctorius* L were tested for antibacterial activity against *Staphylococcus aureus* ATCC 29213, methicillin resistant *S. aureus* ATCC 33591(MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC27853, *Mycobacterium intracellulare* ATCC 23068 and antifungal activity against *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, *Aspergillusfumigatus* ATCC 204305. Ciprofloxacin and Amphotericin-B were used as positive control for bacteria and fungi respectively [18].

Antimalarial Activity: In vitro antimalarial activity was determined against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum* by measuring plasmodial LDH activity. Chloroquine was used as positive control [19].

Antileishmanial Activity: The antileishmanial activity was tested against *Leishmania donovani* promastigotes; pentamidine and Amphotericin-B were used as positive controls [20].

RESULTS AND DISCUSSION

In the present study attention is being paid to medicaments comprising antibiotics composed of extract obtained from medicinal vegetative raw materials method of supercritical carbon dioxide extraction. Advantage of the present method is the use of border states of substances, which are called by fluid, rather than individual of phase states (liquid or gas). As a result, there is an extension of the spectrum of extracted substances, reducing time and process chains (due to the high process speed due to the low density of the medium) and others. Application in medicine to develop a prospectively drugs mean the efficiency and prolongation of the therapeutic effect, a minor amount of adverse effects, the possibility of their wide application in the pediatric, geriatric practice and regenerative medicine applications.

Obtained an experimental industrial series of dental gels based medicinal vegetative raw materials (*Alhagi kirghisorum*) in the Antigen Ltd Research and Production Company.

The Composition of Gels with an Extract of Alhagi: The Composition per 100 g of gel number 1, in grams

Dry an extract of Alhagi	0,50
lidocaine	2,00

Excipients:

Collagen	30,00
hyaluronic acid	0,02

The Composition of Gels with Antibiotic: The Composition per 100 g of gel number 2, in grams

Metronizazol	0,010
Cefuroxime	0,015
Lidocaine	2,000

Excipients:

Collagen	30,00
hyaluronic acid	0,02

Biopharmaceutical research conducted by the release of the active substance from the gels to various bases, resulting in it was established that the release of more complete and rapid is the process basis of collagen compared to gelatin-glycerin and polyethylene glycol basis.

Phytochemical examination of the ethyl acetate and methanol extracts of *Carthamus tinctorius* L. revealed the presence of secondary metabolites. These metabolites include alkaloids, steroids, glycosides and reducing sugars (Table 1). These metabolites are responsible for activity the plant displayed in treatment of skin diseases, wounds as claimed by traditional healers.

Antimicrobial Activity: Antimicrobial study was done for one of the isolated compounds and essential oil against five fungi and five bacteria. The oil was found to have a good activity against *Cryptococcus neoformans* with an IC_{50} value of 8 µg/ml. In articles [19, 20] reported that the essential oil of *Carthamus tinctorius* L exhibited a negative antifungal effect using broth micro dilution and disc gel diffusion methods. The antifungal activity was assessed against five dermatophytes (*Trichophyton mentagrophytes, T.rubrum, Microsporum canis, M. nanum* and *Epidermophyton floccosum*),

Table 1: Phytochemical Study of Ethyl acetate and Methanol extracts of *Carthamus tinctorius* L.

Plant Constituents *Carthamus tinctorius* L	Methanol extract	*Ethyl acetate* extract
Carbohydrate	+	+
Glycosides	+	+
Protein and amino acids	-	+
Alkaloids	-	-
Flavonoids	+	+
Phytosterols	+	+
Tannins and Phenolic	+	+
Saponins	-	-
Steroids	+	+
Suger	+	+

Table 2: Biological activity of ethyl acetate and methanol extraction of *Carthamus tinctorius* L.

Biological activity	Test parasite	Rhizome essential oil (µg/ml)
Antileishmanial activity	*L. donovani* IC_{50}	>80
	IC_{90}	>80
Antimalarial activity	*P.falciparum* D6 IC_{50}	
	W2 IC_{50}	>47600
Antimicrobial	*C.neoformans*	20.41

three filamentous fungi (*Aspergillous niger, A. fumigatus and Mucor sps.*) and five strains of yeast (*Saccharomyces cerevisiae, C. neoformans, Candida albicans, C. tropicalis* and *Torulopsis glabrata*). This report is similar with what we are reporting however we found the essential oil *Carthamus tinctorius* L with IC_{50} against *C. neoformans*.

Antimalarial Activity: Antimalarial activity was studied for oil and a compound zerumbone. The oil showed moderate antimalarial activity with an IC_{50} value equal to 17.5 µg/ml and 20.0 µg/ml against *P. falciparum* D6 and *P. falciparum* W2 respectively. *Carthamus tinctorius* L showed good activity with low IC_{50} values against *P. falciparum* D6 and *P. falciparum* W2 (Table 2) [20].

Antileishmanial Activity: Antileishmanial evaluation was done for the oil and the compound *Carthamus tinctorius* L. The oil showed moderate activity with IC_{50} and IC_{90} values of 4.6 µg/ml and 18.0 µg/ml respectively. Zerumbone showed good activity with low IC_{50} and IC_{90} values (Table 2) [21].

CONCLUSIONS

Obtained an experimental industrial series of dental gels based medicinal vegetative raw materials (*Alhagi kirghisorum*) in the Antigen Ltd Research and Production Company. Development of an optimal composition and rational technology medicinal products based on collagen and hyaluronic acid has antimicrobial, anti-inflammatory, regenerative, curative effect.

From the result obtained in the study, it can be concluded that the extraction of ethyl acetate and methanol, ethyl acetate is more effective extraction from than methanol extract. The traditional use of *Carthamus tinctorius* L against various skin infections wounds has been corroborated since the extracts displayed in vitro antimicrobial properties against different test organisms. The fact that the ethyl acetate extracts of this medicinal plant were very active against the test organisms. To our knowledge from literature, this is the first time, report on the antileishmanial activity, antimalarial activity and antimicrobial properties of extracts. Further studies of other phytoactive compounds will possibly lead to exploration of new methods for therapeutic and industrial application. This will help mankind in short term, by providing improved phytotherapeutic preparations but extensive pharmacological study for elucidation of new drug molecule is needed. This compilation of works is a good source of ethno-botanical, chemical and pharmacological information and usefulness of *Carthamus tinctorius.*

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