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A COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITIES OF ZIZIPHUS AND COLOCYNTH FROM SAUDI ARABIA DESERTS AND PROPOSED PHARMACEUTICAL PRODUCTS

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ABSTRACT

Excess free radicals production plays a significant role in causing many chronic and degenerative diseases. Antioxidants can protect the human body from various ailments by scavenging free radicals or by accelerating their decomposition. This research work evaluated the antioxidant content of methanolic extracts of *Ziziphus spina–christi* leaves, *Citrullus colocynthis* fruits and seeds by UV-spectrophotometry. The affordability and accessibility of these two plants in Saudi Arabian deserts have made them widely used by Saudi population for many different medical conditions. The analysis showed profound concentrations of antioxidant compounds in these extracts and thus prove their abilities as free radical scavengers. Colocynth seeds extracts showed the most flavonoid content. Ziziphus leaves extracts proved to have the highest phenolic content with utmost antioxidant activities (93.6 %) that overweigh that of standard ascorbic acid (87.4%). The extracts of the studied indigenous plants have been formulated in oral and topical pharmaceutical dosage forms with the appropriate antioxidant doses that are beyond toxicity levels.

Keywords: Ziziphus; Colocynth; antioxidants; UV spectrophotometer; pharmaceuticals.

INTRODUCTION

Traditional medicine, which is based on theories, beliefs and personal experiences, is a pool of knowledge skills and practices that is being used by various cultures for maintaining health, preventing, diagnosing, improving and treating both physical and psychological illnesses [1]. The affordability and accessibility of the medicinal plants have made them an important part of many people's life all over the world [2]. Many herbs and roots are used in the Kingdom of Saudi Arabia by common people for various ailments [3].

Free radicals contain unpaired electrons spinning on their outer shells around the nucleus [4]. Along with the reactive oxygen species (ROS), they are the main sources for the primary catalyst which induces oxidation and oxidative stress that causes many diseases [5,6]. There are endogenous and exogenous sources of free radicals in the body. The endogenous sources include nutrient metabolism and aging processes while exogenous sources may include; tobacco smoking, ionizing radiation, air pollution, organic solvents and pesticides [7]. Reactive oxygen species alter DNA causing mutation and chromosomal damage and leading to the development of chronic diseases [8,9]. Synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoloune (BHT) have been used extensively as antioxidants and food preservatives, but they may possibly be carcinogenic and toxic[10]. In this regard, researchers have focused on medicinal plants as natural and cheap antioxidant substitute as these phytochemicals can scavenge free radicals and prevent the transmission of their reactions and thus protect the human body from their damages [10-11]. *Ziziphus spina–christi* (L.), of Rhamnaceae family, is one of the five species belonging to the genus Ziziphus which are native to Saudi Arabia.

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It is commonly known in Arabic as Siddir or Nubak[12-13]. It is a shrub or a tall tree that might reach 20 meters in height. Its leaves are glabrous on upper surface, finely pubescent on the lower one, ellipsoid or ovate lanceolate with obtuse or acute apex [13]. Ziziphus tree is greatly respected by Muslims since it was mentioned in Sunnah and the Holly Quraan twice (LIII:13-18; LVI: 28-32) [14]. The leaves of Ziziphus show the presence of saponins, flavonoids, alkaloids, triterpenoids, glycosides, and other phenolic compounds[1]. Ziziphus has been used for treatment of many diseases; such as weaknesses, diabetes, pharyngitis, bronchitis, anemia, diarrhea, skin infections, fever, sleep irritability, liver problems, digestive disorders and many others [15]. In addition, it has antimicrobial, anti-inflammatory and antiulcer properties and has been used for the treatment of cancer and cardiovascular disorders [16-25].

Citrullus colocynthis (L.) is a medicinal plant species of Cucurbitaceae family which is known in Arabic as Bitter Apple or Handal. It is widespread in different parts of Saudi Arabia as it grows rapidly in sandy soil [26]. It is a common annual wild procumbent plant with simple tendrils, small yellow flowers and very bitter fruits [27]. Fruit is subglobose, slightly depressed, 4-10 cm in diameter, variegated with green and yellow stripes, becoming glabrous, creamy-yellow and with a dry, spongy pulp when ripe [28]. Seeds are obovate, dark brown and about 7.5 mm long [28]. Colocynthis was reported to have many constituents such as alkaloids, flavonoids, saponins, tannins, carbohydrates, glycosides, and essential oils [29]. The ethanobotanical uses of colocynthis includes its application as an abortifacient, cathartic, purgative, and vermifuge, and as medications for fever, cancer, amenorrhea, jaundice, leukemia, rheumatism and tumor [30,31].

Due to the antioxidant activity of naturally occurring substances in some plants, attention has increased on the protective activities of these natural compounds against chronic disorders caused by oxidative processes [32]. Colocynth and Ziziphus are two indigenous plants in Saudi deserts and have been used traditionally by Saudi population either as fresh herbs or as an infusion. Both plants have many compounds that possess free radical scavenging properties. Therefore, this study was directed to the evaluation of their antioxidant activities by spectrophotometric assay methods. Formulation of their extracts in certain dosage forms will render their therapeutic uses more efficient and safe.

AIM OF THE STUDY

The main objectives of this study have been directed to;

1- Evaluate antioxidant activities of *Ziziphus spina–christi* leaves (ZSL), and *Citrullus colocynth* seeds (CCS) and fruits (CCF) collected from Saudi deserts.

2- Correlate their antioxidant contents with their free radical scavenging properties.

3- Formulate pharmaceutical products from those extracts in the appropriate doses.

MATERIALS AND METHODS

MATERIALS

Ziziphus spina–christi leaves and *Citrullus colocynth* fruits, Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, aluminum chloride, ascorbic acid, gallic acid, catechins, sodium nitrite, sodium hydroxide, ethanol and methanol.

INSTRUMENTS

- Soxhlet Apparatus
- Rotary Evaporator (Butchi-R-215)
- Double Beam UV-Visible Spectrophotometer (DBUVS) (Thermo-UVZ165008)

SAMPLE COLLECTION, DRYING, AND GRINDING

Fresh samples of Ziziphus leaves and Colocynth fruits were collected from Saudi deserts (Al-Kharj) in winter. Colocynth seeds were carefully separated from the fruit's bulb. The samples were air-dried at room temperature in the laboratory fume hood. Ziziphus leaves, Colocynth seeds and fruits (seeds-free) were ground separately in a mixer-grinder into a fine powder.

EXTRACTION

Samples were extracted using Soxhlet apparatus adjusted to 70° C. In a patch, 10 g of each sample were extracted with 200 ml methanol in replicates until the produced extract becomes clear (colorless). The collected methanolic extracts were evaporated to dryness in a rotary evaporator and weighed. For each plant, 500 g were extracted, and the residue of each was weighed, tightly covered and stored in the fridge at 4°C.

TOTAL PHENOLIC COMPOUNDS ASSAY

The total phenolic contents of ZSL, CCS and CCF extracts were determined using Folin Ciocalteu's reagent. 1 ml of the reagent (0.1 N) was added to 1 ml of standard gallic acid/sample solutions and mixed thoroughly. 4 ml of sodium carbonate (7.5 % (w/v)) was added, and the reaction mixture was completed to 10 ml with distilled water. The mixture was warmed in a water bath at 35° C for 5 min. Subsequently, contents were centrifuged at 2000 RPM for 5 min and the absorbance (Abs) of the supernatant was measured at 760 nm using DBUVS. Each concentration of (gallic acid and ZSL, 50 - 10^3 µg/ml (w/v)) and (CCF and CCS, 10^3 - 10^4 µg/ml (w/v)) was prepared and measured in triplicates.

Total phenolic content was calculated as a percentage of gallic acid (%w/w) per 100 g sample.

TOTAL FLAVONOID ASSAY

Total flavonoid contents were measured with Aluminum chloride- colorimetric assay [33]. 1 ml of each of catechins, ZSL, CCS and CCF solutions was added to 4 ml of distilled water and 0.3 ml of sodium nitrite (5% (w/v)). After 5 minutes, 0.3 ml of aluminum chloride (10% (w/v)) was added. After 6 min, 2 ml of 1 mol/L sodium hydroxide were added, and the total volume was made up to 10 ml with distilled water. Each reaction mixture solution was mixed well, and the absorbance was measured at 510 nm using DBUVS. Each sample concentration (20 - 10^3 µg/ml (w/v)) and catechins (2.5 - 300 µg/ml (w/v)) was done in triplicates. Total flavonoid content was calculated as a percentage of catechins (% w/w) per 100 g sample.

DPPH FREE RADICAL SCAVENGING ACTIVITY ASSAY

The percentage of antioxidant activity (%AA) of each extract was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture was prepared by adding 0.5 ml of sample (1 g % (w/v)), 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution (0.125 mM) in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. Abs or changes in color from deep purple to light yellow was read at 517 nm after 100 min of reaction using DBUVS. The blank mixture consisted of 3.3 ml ethanol and 0.5 ml sample while the control solution was prepared by mixing 3.5 ml ethanol and 0.3 ml DPPH radical solution. The scavenging/antioxidant activity percentage (%AA) was calculated according to the following formula [34];

$$\%AA = 100 - \left[\frac{\left(\mathrm{Abs}_{\mathrm{Sample}} - \mathrm{Abs}_{\mathrm{Blank}}\right) \times 100}{\mathrm{Abs}_{\mathrm{Control}}}\right]$$

PHARMACEUTICAL PREPARATIONS
ORAL DOSAGE FORMS

ZSL and CCF extracts' residues were completely dissolved in ethanol then starch was added and mixed over 40° C until complete evaporation of ethanol.

The obtained paste was further dried in an oven at 40° C for half an hour and left overnight in a desiccator. ZSL- and CCF-starch mixtures were mixed with lactose as diluent and their bulk densities were measured (0.571 and 0.455), respectively. Each was then filled into a capsule body shell (size 00) so each capsule contained 300 mg of ZSL and 25 mg of CCF extracts. Finally, the capsules were polished using sodium chloride, packed and labeled.

TOPICAL DOSAGE FORMS

100 g cream were formulated from each of the studied extracts using borax, span 80, liquid paraffin, carnauba wax, beeswax, water and rose oil for fragrance so that the cream contained 1 g % (w/v) of ZSL and 3 g % (w/v) of CCF and CCS extracts.

RESULTS AND DISCUSSION

The antioxidant activities of selected plants were evaluated and compared spectrophotometrically (Figures 1-3). The percentages of yield, total phenolic compounds and total flavonoids are shown in table 1. The UV-spectrophotometry results showed that ZSL had the highest content of phenolic compounds (62.9 % (w/w)) calculated as gallic acid equivalent per 100 g sample which was eight- and thirteen-fold more than CCF and CCS, respectively (Table 1). On the other hand, CCS showed the highest content of flavonoids (33.4 % (w/w)) calculated as catechins equivalent per 100 g sample which was three- and seven-fold more than ZSL and CCF, respectively (Table 1).Our findings showed that the calculated free radical scavenging activity percentage (%AA) of ZSL crude extract overweight that of the standard pure ascorbic acid by 6.2 % (Table 2). These findings were consistent with the results of Folin-ciocalteu's assay where ZSL showed the highest content of phenolic compounds (Table 1). Although CCS did not show the highest content of phenolic compounds, it was found to have the highest content of flavonoids and potent free radical scavenging properties which was closely comparable to that of ascorbic acid (Tables 1-2). Literature stated the recommended daily allowance (RDA) of vitamin C (ascorbic acid) for adults of both genders to be 60 mg, while no records of RDA of the powerful antioxidants; catechins or gallic acid derivatives [35].

Table 1: The percentages of yield, gallic acid- and catechins-equivalents of the studied extracts as calculated per 100 g samples using Folin-ciocalteu's and Aluminum-chloride colorimetric assay methods

Percentage (% w/w)	CCF	CCS	ZSL
Yield	19.6	18	**20.4**
Gallic acid equivalent	7.8	4.7	**62.9**
Catechins equivalent	4.3	**33.4**	10.1

Table 2: UV-spectrophotometer absorbance of ZSL, CCS, CCF and ascorbic acid as determined by DPPH-free radical scavenging assay at 517 nm and the calculated %AA

Absorbance	Ascorbic acid *	CCF*	CCS*	ZSL*
Sample	1.021	0.756	0.148	0.392
Blank	0.756	0.179	0.005	0.326
Control	0.931	0.931	0.931	0.931
%AA	87.4 %	43.4 %	86.0 %	**93.6 %**

* Concentration is 1g% (w/v)

Figure 1: UV-spectrophotometer graph of the total phenolic assay of ZSL using gallic acid as a standard by Folin-ciocalteu's method at 760 nm (Y=1526.5X-13.43, R^2 = 0.9998)

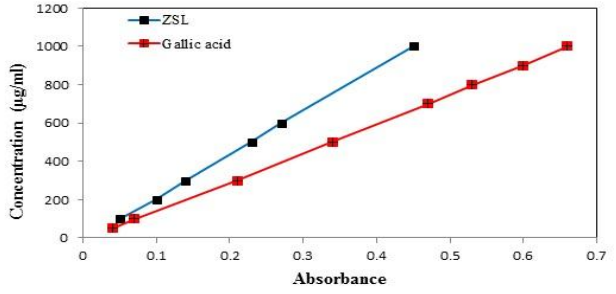


Figure 2: UV-spectrophotometer graph of the total phenolic assay of CCS and CCF by Folin-ciocalteu's method at 760 nm(Y=1526.5X-13.43, R^2 = 0.9998)

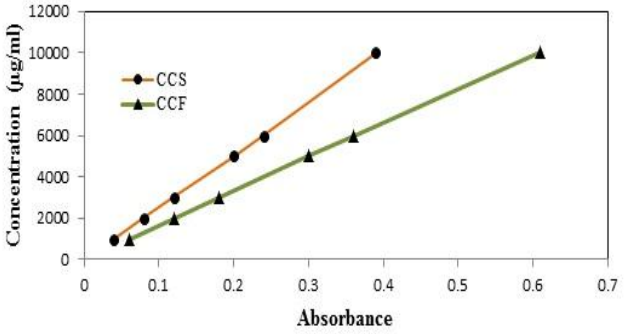
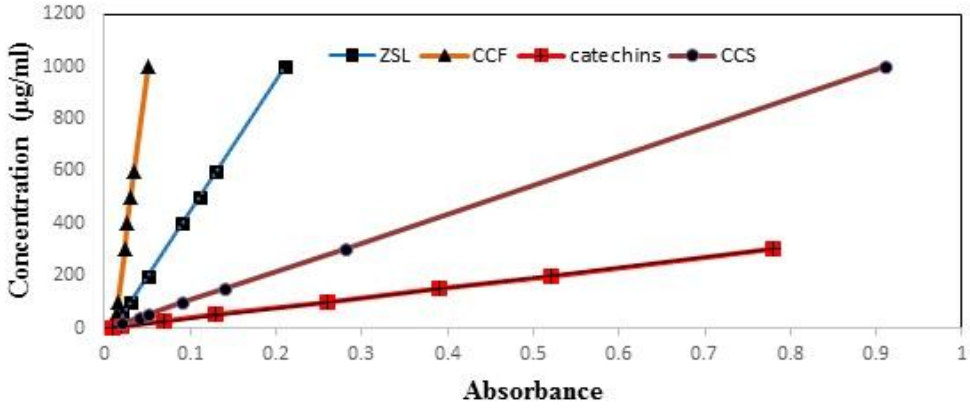


Figure 3: UV-spectrophotometer graph of the total flavonoid assay of ZSL, CCF and CCS using catechins as a standard by Aluminium Chloride-colorimetric method at 510 nm (Y= 387.32X-1.4811, R^2 = 0.9999)



Traditionally, Saudi populations used to consume Handal seeds (CCS) as snacks for its laxative and hypoglycemic properties while the fruit's bulb is not frequently used due to its intense bitterness. They also topically applied the infusion of Siddir (ZSL) for wound healing. This study provided CCF and ZSL capsules formulated as 25mg/cap and 300 mg/cap, respectively. The proposed dosage is four capsules per day for a healthy adult. These capsules were formulated to provide a safe antioxidant/anti-aging natural dietary supplement within suitable doses without any toxicity and minimal side effects. Although oral LD_{50} of ZSL extracts was as high as 3820 mg/kg in studies that were made on mice [36], the literature indicated that as little as 1.5 tea spoonful of CCF powder was potentially fatal in animal studies [37]. Formulating ZSL extract (with the best free radical scavenging activities) as 1 g % (w/v) cream would provide a convenient and safe formula for wound healing with better spread-ability and skin absorbability than the traditional infusion. The proposed topical formulas that were made out of these extracts can be used safely for anti-aging (anti-wrinkles) effects on daily bases without the potential health hazards upon using synthetic ones [10].

CONCLUSION

All studied plant extracts were found to have antioxidant constituents in considerable concentrations. These plants are available in Saudi deserts almost all year long as they grow wildly with negligible cultivation costs.

Therefore, they are considered as cheap and easily-accessible sources of natural antioxidant compounds.

Oral formulations of Ziziphus and Colocynth would be suitable dietary supplements with acceptable doses and bitter taste-free. On the other hand, the formulated creams would provide natural and safe substitutes to anti-wrinkles formulas that are currently available in the market with possible carcinogenic properties.

The authors suggest introducing the formulated dosage forms of the studied plant extracts to pre-clinical and further clinical trials for processing of FDA approval since such products are not available in the pharmaceuticals market yet. The daily use of these plant extracts in the appropriate doses (formulated pharmaceutical preparations) can be necessary in the future to prevent degenerative and/or chronic diseases that are caused by free radicals.

To the best of our knowledge, this is the first report of a comparative study of the antioxidant activities of Ziziphus leaves, Colocynth seeds and fruits collected from Saudi deserts with proposed oral and topical pharmaceutical products. These indigenous plants cost little and deserve more attention from researchers to formulate varieties of pharmaceutical products of their extracts. The latter can greatly improve the general health and help in the prophylaxis of many ailments.

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DECLARATION OF INTEREST

There are no conflicts of interests to be reported.

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