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PHARMACOGNOSTIC STUDIES ON *COFFEE ARABICA* L. HUSKS: A BRILLIANT SOURCE OF ANTIOXIDANT AGENTS

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ABSTRACT

The current study was conducted to determine standardization profile of *Coffee Arabica* L. is not study before. *Coffee Arabica* L. (Rubiaceae), is native to South America and south west Asia. Coffee pulps and husks are rich in caffeine, tannins, phenolic compounds, and other organic matter and carbohydrate. Among these compounds found in coffee pulp and husk, phenolic compounds are known to be a potent inhibitor of fermentation. Phenolic compounds cause loss of integrity of the biological membranes of microorganisms such as *Saccharomyces cerevisiae*, affecting the selective barrier capacity of membranes and the enzymatic matrix of microorganisms. These inhibitory compounds include alcohols, aldehydes, ketones, and acids. Pharmacognostical and preliminary phytochemical screening of *Coffee arabica*L husk is going to be helpful to authenticate and avoid substitution as well as adulteration in the raw material. The microscopic characters, physiochemical information and FTIR will be useful in the expansion of monograph.

KEYWARDS: *Coffee Arabica*, macro&microscopy, UV& FTIR analysis, antioxidants.

INTRODUCTION

Coffee tree is one of the most substantial agricultural goods globally. *Coffea Arabica* L. and *Coffea robusta* are F. Rubiaceae, both fundamental varieties of the genus cultivated worldwide for trade production. It is represent in Saudi Arabia by one species *Coffea Arabica* L., SP, Pl. (1753). Plate Rub. 15 b (Migahid 1978 & Chaudhary 2001). *Coffea arabica* L is widespread especially in South region of Saudi Arabia and in Kholan bin Amer region, AbdulAziz Al-Melahe Farm, 2 Km, Sana'a, Yamane. Pandey et al., (Pandey *et al*., 2000), classified the methods of processing coffee into wet and dry processes that result from these processes are called coffee pulp and husk, respectively. The coffee husk (CH) and pulp (CP) have similar compositions; they contain 7.5–15 % protein, 2.0– 7.0 % fat, and 21–32 % carbohydrates (Ulloa Rojas 2003). The CP and CH components may vary based on the processing mode, soil type and cultivar (Pandey *et al*., 2000). CPs and CHs are rich in caffeine, tannins, phenolic compounds, and other organic matter.

The antioxidant (AO) activity of polyphenolics is said to be due to their ability to sweep highly reactive radicals (Balasundram 2006). Naturalistic AOs from vegetables and fruits supply a protection that delays the process of oxidative damage (Jacob and Burri, 1996). Numerous reports have shown that abundant of polyphenols and flavonoids participate significantly to the overall AO activity of multiple vegetables and fruits (Luo *et al*., 2002; Vinson 1999).

Some synthetic AOs, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and tertbutyl hydroquinone (TBHQ), have been used excessively as AOs in foods, but concerns above the safety of use in addition to rise manufacturing expenses and minimize efficiency of synthetic AO when compared to natural ones (Balasundram, 2006). Now, the natural AOs commercially recruitment contain ascorbic acid, tocopherols, and many herb extracts from sage, rosemary, and green tea (Djarmati *et al*., 1991; Ramarathnam *et al*., 1995; Tena *et al*., 1997; Yoshida *et al*., 1999).

Traditionally, CH is used as fermented for tea, named coffee cherry tea (Pabari 2014), *cascara*, *sultana*, *qishr*or*buno* (Baldwin, 2010; Baldwin 2009; Wiser 2011), the name based on which region the CH are fermented. The CH is fermented alone or with cinnamon. Also, could be sell the ground CH as a food supplementary for consume in granolas, smoothies, and juices (Turkyilmaz 2013). A further, the CH might also be commence as allergic-friendly, owing to its naturally gluten free (Esatbeyoglu 2015). The taste is characterized like fruity, such as blackcurrant and watermelon (Pabari 2014) to strawberries and raisins (Wiser 2011).

Among the compounds found in coffee pulp and husk, phenolic compounds are known to be a potent inhibitor of fermentation (Klinke 2004). Phenolic compounds cause loss of integrity of the biological membranes of microorganisms such as *Saccharomyces cerevisiae*, affecting the selective barrier capacity of membranes and the enzymatic matrix of microorganisms (Heipieper *et al*., 1994; Palmqvist *et al*., 2013). All of these inhibitory components contain ketones, aldehydes, alcohols, and acids (Almeida *et al*., 2007). Phenolic compounds of low molecular weight are more toxic to microorganisms than the phenolic compound of high molecular weight (Larsson *et al*., 2000). Literature survey clearly revealed that coffee husks products from agro and industrial wastes growing in south region of the Saudi Arabia have not been subjected to any pharmacognostical studies. In our study we focus in using agro- and food industry wastes that can be utilized as valuable and cheap sources of antioxidants and free radical scavenging compounds. We have chosen the waste products one of important plants growing in south region of Saudi Arabia and produced in large quantity which is coffee husk (*Coffea arabica*). Therefore it was of interest to carry out a pharmacognostical investigation with the aim of establish standardization profile of *Coffee arabica* Husks as well as a qualitative identification of chemical constituents that possesses antioxidants activities.

MATERIAL AND METHODS

Plant material

The *Coffea Arabica* L., SP, Pl. (1753). Plate Rub. 15 b. Husk was collected in Feb 2016 from Kholan bin Amer region, AbdulAziz Al-Melahe Farm, 2 Km, Sana'a, Yamane. The plant material was identified by Dr. Mahmoud AbdulAziz Mahmoud, College of Food & Agriculture Sciences voucher specimen (PDH#437) was kept in department of pharmacognosy, college of pharmacy, KSU.

Preparation of extract

The air dried coarse powdered coffee husk (1.85 kg) was extracted by maceration with 95% ethanol at room temperature. After filtration and evaporation of the alcohol using rotary vacuum at 45ºC, the combined alcoholic extract was dark gummy extract (153.7 g, yield 8.31 % w/w, coded as COH). A part of solvent free COH was underwent to phytochemical preliminary screening qualitative tests by handling different reagents for the determination of numerous classes of active components (Wagner and Bladt 1996).

Preparation of botanical study

Macroscopic evaluation

Macro morphological characters of the *Coffea Arabica* L Husk(color, taste, odor and appearance) were studied and noted.

Microscopic evaluation

Transverse section

Coffee husks were prepared according to a procedure described by Abdulla Al-dea'ajy with some modifications (Abdulla *et al*., 1997). This procedure was done by using different steps such as: Fixation, Dehydration, Clearing, Infiltration, Embedding, Trimming, Sectioning, and Staining. Fixation. Fixation is a discontinuation of normal life actions in the tissue and establishing of the structure of the tissue. The aim of fixation is to maintain structure as possible compared to the living case by using formalin-aceto-alcohol (FAA), (95 ml of alcohol, 5 ml of formalin, 5 ml of glacial acetic acid). The coffee husk tissues were soaked in FAA in glass sealed container over 24 hrs. Dehydration. Dehydration is the chemical dehydration of the specimen. Common removal of water are alcohol and/or acetone in the following water removing steps, Alcohol 80%, Alcohol 90%, Alcohol 100%, and Alcohol 100% for 2 hrs, 2hrs, 2hrs and 24 hrs respectively. Clearing. Clearing is a process that consists of replacing the dehydrator by a material that would be miscible with the embedding medium like paraffin. The majority of clearing agent is xylene. Xylene is used with ethanol with different ratios in the following clearing steps, xylene:ethanol 1:3, 1:1, 3:1, and xylene only for two hours each. Infiltration. Infiltration is a process that increases the concentration of paraffin by reading paraffin to the tissue again with xylene and when the paraffin is melted, adds paraffin again for 24 – 48 hours. Embedding. The tissue is ready to be located in a mold and cooled. The choice of mold will depend on the type of chuck in the microtome device. There are various kinds of mold can be handled. Spray a stainless steel mold with liberation compound then decant a little amount melted Paraplast (fresh) into it. Transmit the tissue with hot forceps and place it in the center of the depression. Then put the white plastic form on top of the mold and replenish with melted paraplast. Cool the upper exterior surface of the Paraplast by blowing kindly on it. Tissues at this case are very fragile. When a foam of paraplast has formed on the upper surface, sink the cup kindly into a cold water bath. The block would be damaged if it is submerged before it's top has created a protective foam. Cool completely in cold tap water. Paraplast normally divides in the line of least resistance-right meanwhile the tissue. Transverse section (T.S) of the coffee husk were prepared using Microtome device (microTec®), carried in college of Science, KSU, Riyadh.

Study of transverse section cellular arrangement of the coffee husk was examined after making slide of thin T.S of the husk. The complete histology was observed under electronic microscope (leica-microsystem, Schweiz, AG, CH-9435 Heerbrugg) (Shruthi *et al*., 2010).

Study of powder characteristics

Fine powder of the *Coffea Arabica* L Husk was used for powder microscopy. The sample was separately treated with 10% chloral hydrate, 50% glycerin and 5% iodine solution (Iyengar 1974).

Physio-chemical analysis

For the determination of physio-chemical parameters, moisture content as well as total ash, acid insoluble ash and water soluble ash values of husk coarse powdered were done as described in the British Pharmacopeia (Migahid 1989).

Moisture content

Moisture content of husk powder was determined by weighing 2 g of powder sample in a silica crucible and then placed the silica crucible in stove at 105°C for a period till fixed weight of sample was determined.

% of moisture content = weight loss of sample/ Weight of sample ×100

Total Ash value

Certain weight of the coffee husk (4 g)is incinerated in a silica crucible by gradually increased the temperature and repeat this step until constant weight was obtained. Total ash is mainly composed of oxides and carbonates of Ca, Mg, Na, K and Silicon.

%of total ash value = Total ash weight/ sample weight ×100

Acid-insoluble ash

Certain weight of the coffee husk (4 g) is incinerated in a silica crucible, then the ash is dissolved in dilute mineral acid (H_2SO_4) filtered and dried. The dried residue is weighted and the acid-insoluble ash is calculated. The ash dissolved in acid contains mainly of oxides and carbonate of Ca, Na, K, and is termed acid insoluble ash which is calculated by difference.

Water soluble ash

Certain weight of the coffee husk (4 g) is burnt in a crucible silica then the ash is dissolved in water, filtered and dried. The dried residue is weighted and the water-soluble ash is calculated by difference. It consists mainly of oxides and carbonates of sodium and potassium.

Fluorescence analysis

The fluorescence behavior of the coffee husk powder in the visible light and UV light were carried out by soaking the powder in different reagent solutions and viewing under the light of short and long wavelength in a UV chamber Wallis 1967; Anonymous 2002).

FTIR Analysis

Coffee husk fine powder was used for FTIR analysis. Infrared spectra were recorded on a Perkin Eimer FTIR Spectrometer (spectrum BX) 8000 series, between 4,000-400cm^{-1}.

RESULTS

Phytochemical screening

Phytochemical screening of alcoholic extract of coffee husksshowed the presence of Alkaloids, Carbohydrates and /or glycoside, Flavonoids, Saponins, Triterenes/sterol, Tannins, and Volatile constituents. Anthraquinones and Cardiac glycosides are absent in coffee husk alcoholic extract, table 1.

Table 1: The results of preliminary phytochemical screening of the powder of coffee husk alcoholic extract.

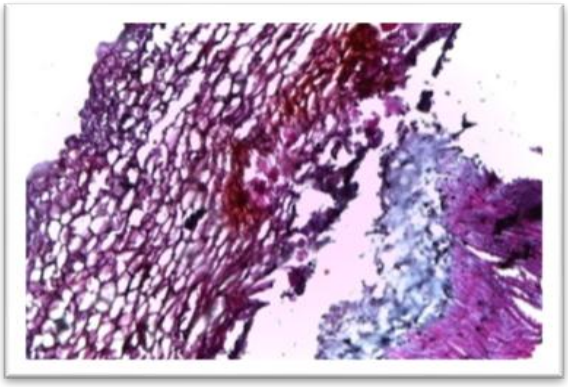
Active constituents	Results
Alkaloids	+
Carbohydrates and /or glycoside	+
Anthraquinones	-
Cardiac glycosides	-
Flavonoids	+
Saponins	+
Triterenes/sterol	+
Tannins	+
Volatile constituents	+

Macroscopic evaluation

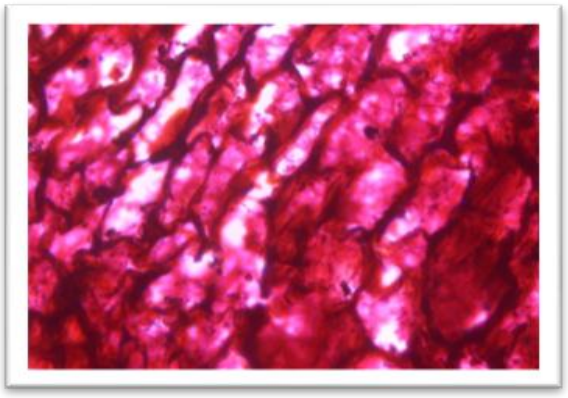
Coffee husk is shrub or a small tree, leaves opposite or in whorls of 3's, elliptic-ovate, up to c.20x8cm, glossy, glabrous, entire, acute or acuminate. Flowers are white, fragrant, in the axillary clusters of 2-9 flowers. Corolla5-lobed, the lobes spreading, c.18 mm long, longer than the tube, Stigma 2-lobed. Berry c.13 mm long, crimson red, 2-seeded.

Microscopic evaluation

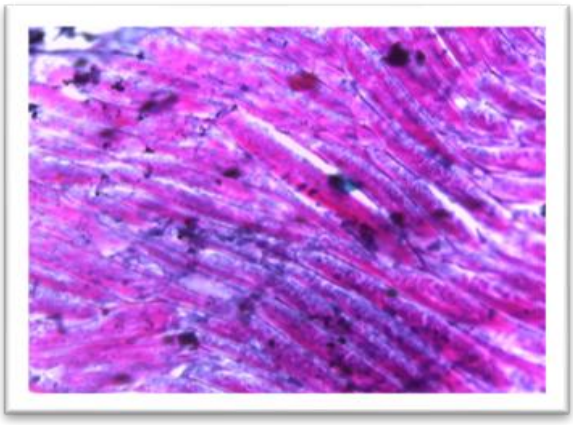
Study of husk transverse section of *C. Arabica* showed the epicarp cells are wide and polygonal. The next layer is the mesocarp, the cells are bigger and more regular in outline than the epicarp. Fibrovascular bundles are scattered through the compressed cells of the mesocarp. The cell walls are large, thick, amorphous, and dark masses as well as large crystals are found within the cell and on the surface of the layer respectively. The vascular bundles contain mainly of wood fibers, bast and vessels. Spiral and pitted vessels are also present. Inside the mesocarp is a thin layer of endocarp called parchment. The parenchyma (palisade cells) is an elongated, a thin-walled tissue. The endosperm, the CP, is covered with a spermaderm named silver skin. The bean contains of two hemispheres with flattening neighboring sides. Each bean has an inside layer of silver skin while the parchment both covers the spheres and separated them from each other, fig. 1.



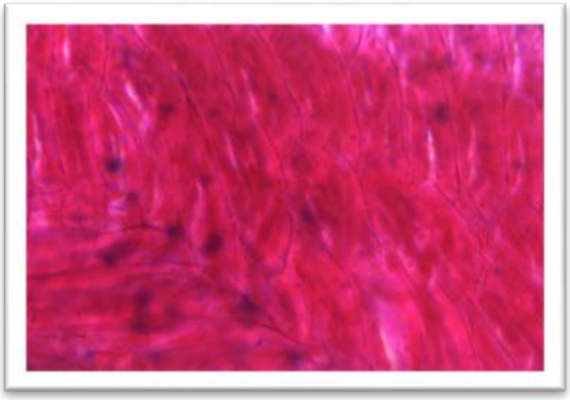
A



B



D



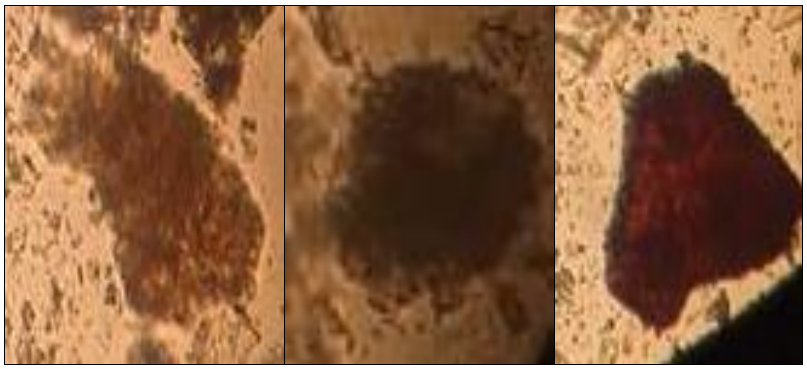
C

Fig. 1: Transverse section of the coffee husk, A x10, B-D x 40.

Study of powder elements

The powder microscopy of husk of *C. Arabica* in 10% chloral hydrate, 50% glycerin and 5% iodine solution reagents that showed the presence of wood fiber, oleo resin masses, glandular trichomes, fragments of palisade cell, epidermal cell, simple and compounds of starch granules and clustered fiber sheets of calcium oxalate, fig. 2.

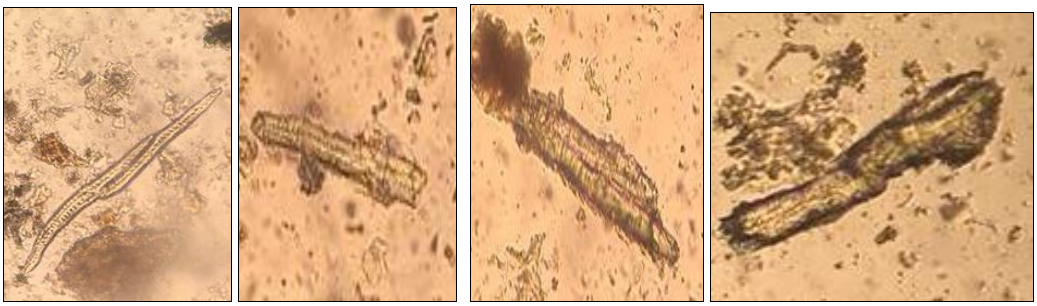
Starch granules



Oleo resin masses

Cluster crystals sheet of Caox

Glandular trichomes



Fibers **Woody fiber**

Fig. 2: The powder of *C. arabica* husk.

Physiochemical analysis

For the detection of adulteration in the powdered husk physiochemical is the important parameter. Moisture content as well as total ash, acid insoluble ash and water soluble ash values were illustrated in table 2.

Table 2: Physiochemical analysis of powdered husk of *C.arabica.*

The Pharmacopoeial constant	Value (%w/w)
Total ash	0.65
Acid insoluble ash	0.48
Water soluble ash	0.18
Moisture contents	0.015

n=3 (dry weight bases)

Fluorescence analysis The characteristic fluorescent colors emitted by the husk powder after mixing with various reagents under visible and U.V light (short and long) were noted and recorded that are tabulated in table 3.

Table 3: Fluorescence analysis of husk powder of *C.arabica.*

Husk powdered/reagents	Visible light	UV F_{245}	UV F_{366}
Crud powder	Olive green	Olive green	White
Powder+1N NaOH	Reddish brown	Olive green	Yellow
Powder+Picric acid	Florescent green	Dark yellow	Light brawn
Powder+50%H_2SO_4	Light yellow	Light yellow	White
Powder+INHCl	Light brawn	Olive green	Light green
Powder+50%HNO_3	Light yellow	Light green	Light green
Powder+ Acetone	Yellow	Dark yellow	Pink
Powder+5%iodine	Light yellow	Dark yellow	White

FTIR analysis

IR spectrum of *C. arabica* was shown in fig. 3 which manifests prominent transmittance presented at 3400, 2937,2370, 1641, 1401, 1062 and 613cm^{-1}. 3400cm^{-1}peakvalues attributes the presence of saturated C-H stretching while transmittance at 1641 and 1062 cm^{-1} indicates C=O and C-O respectively.

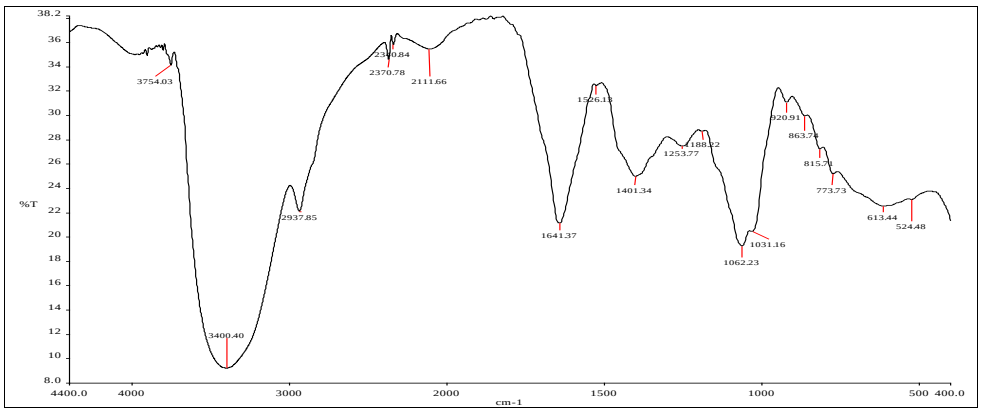


Fig. 3: FTIR Spectra of husk alcoholic extract.

DISCUSSION

The procedure of standardization may be carried out by various pharmacognostic methods. These methods help in authentication and identification of the plant sample. Such information may act as reference data for correct identification and determination of particular sample and also will be helpful in making a monograph of the sample. Moreover, it will perform as a tool to identify substituent and adulterants and will keep in maintaining the quality standard ,reproducibility and efficacy of natural source. The results of the moisture content in *C. Arabica husk* was not high 0.015% that indicates less chances of spoilage of the sample during storage, the excess moisture lead to breakdown of valuable constituents by enzymatic action and may also encourage the growth of fungi during storage (Migahid 1989). The total ash value in *C. Arabica* was 0.65 %, since the accepted range was 22%, which display that the sample has normal complexes of inorganic and organic component (Antia *et al.*, 2006). The high ash value is the indicator of the mineral contents in the food materials (Antia *et al*., 2006) For the identification of functional groups in the *C. arabica* husk FTIR technique was applied to ensure the reactivity of sample towards efficacy and therapeutic action that disclose an important role in estimation of drugs. FTIR is more sensitive and selective experiment than colorimetric experiment and also a time saving technique to analyze and characterized microorganism (Kogel 2000; Grube *et al*., 2008).

CONCLUSION

Pharmacognostical and preliminary phytochemical screening of *Coffee Arabica* husk will be helpful to standardize, authenticate, and to avoid any adulteration and substitution in the raw material. Microscopic characters, physico-chemical information and FTIR spectra will be useful in the expansion of a monograph regarding antioxidant research.

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