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COMPARATIVE STUDY AS ANTIOXIDANT, ANTIMICROBIAL ACTIVITIES AND TOTAL PHENOLIC CONTENT BETWEEN VARIOUS PARTS OF POMEGRANATE

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ABSTRACT: The function of medicinal plants in raise the strength of animal health to overcome with the detestable and difficult position is well certified from old times till time commonality over the world. The present study aims to analyze the *in-vitro* total phenolic content, anti-microbial and antioxidant activities of ethanol extract of three parts of Pomegranate. The antioxidant activity was evaluated by DPPH and total phenolic content using the Folin-Ciocalteu method. The anti-microbial activity determined by the agar well-diffusion method. The results confirm the role of the Pomegranate parts as promising total phenolic content, free radical scavengers, potent antioxidants and anti-microbial agents.

KEYWORDS: Pomegranate peels, albedo, arils, Antioxidant and Antimicrobial activity.

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1.INTRODUCTION

Medicinal plant lives are rich people reference of traditional herbal medicine around the world. Most of the plant's therapeutic properties are due to the presence of secondary bioactive compounds [14]. Pomegranate (Genus *Punica granatum L.*) fruits are widely consumed, fresh and in commercial products, such as succus, jams, and wines. Most parts of pomegranate fruit are known to haved essential antioxidant bodily process. The flower, seed oil, seed infusion, and Peel extract of

pomegranate also have a potent antioxidant activity. Biological activities and pharmaceutical, effects of its various parts (flowers, leaves,arils, and peels) and products (fresh or sweat juices) [15; 6; 12] given its high content of polyphenols such as tannins, ellagic acid, flavonoid , anthocyanins, fatty acids, sterols, terpenoids and alkaloid [31; 26]. Moreover, the extraction of methanol-water for peel pomegranate keep antimicrobial activity against many genus of bacteria and fungi, such as *Listeria monocytogenes, Bacillus subtilis, Staphylococcus aureus, Yersinia enterocolitica, Candida utilize*, *Saccharomyces cerevisiae*, and *Aspergillus niger* 4. Amir [3] reported that the methanol extracts of whole fruits *for P. granatum* were able to inhibit not only growth of *S. aureus* FRI 722 but also the producing anther enterotoxins. The antibacterial activity of arils pomegranate have declared using many studies. Recent, pomegranate aril and peel extracts have a successful antimicrobial activity, as prove by the inhibitory impact on the bacterial development of two imperative human pathogens, including *Staphylococcus aureus* and *Escherichia coli*, often found in food disease [2; 25]. Later the antimicrobial activity of extracts of pomegranate peel and juice on cariogenic bacteria has been also highlighted by Ferrazzano [11]. Moreover Miklavcic Visnjevec [20] have reported the ethanol and water extracts of pomegranate exocarp and mesocarp showed the greatest antimicrobial activity against many bacterial strains tested. In all these studies the sharing of phyto-compounds in the concentrates including phenols, tannins and flavonoids as significant dynamic constituents that might be in charge of these activities. However, research studies dealing with volatiles components of pomegranate and evoking their antibacterial activity are almost absent, except a publication of Mekni [18]. This incited us to build up this investigation to clearness the aroma profile of arils, albedo and peel ethanol extracts of pomegranate varieties and to determine the antimicrobial activity by agar-well diffusion and broth-dilution method against four food-related bacteria two Gram-positive bacteria (*B. subtilis* and *S. aureus*), two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), yeast (*C. albicans*) and fungi *A.flaves* and *A. niger*). While quantifying phenolic (total phenolic content and antioxidant scavenging activity were analyzed.

2. MATERIALS AND METHODS

Preparation of pomegranate's ethanol extracts

Mature Pomegranate fruit were purchased from local markets in Saudi Arabia. The places of these parts were analyzed in table (1). After opening the fruit, the arils (with seeds) were manually separated from the peels. Collected arils, albedo and peels were then rinsed with tap water. These peels, albedo and arils were ground separately in a blender. Fifty grams of blended arils, albedo or peels were placed in 250-ml Erlenmeyer flasks, followed by adding 100 ml of 95% ethanol [10].

Table 1: The places parts of Pomegranate

parts	place
Aril	The botanical term for a seed surrounded by a juice sac
Albedo	The white, fleshy substance directly under the skin of a pomegranate
peel	Husk of a pomegranate. Much of the antioxidant content of the juice comes from crushing the whole fruit

The flasks were then shaken at room temperature for 18 h prior to filtration. The filtrates were concentrated under reduced pressure with a rotary evaporator at 40 °C. All extracts were kept at -20°C in the dark for further use.

Phytochemical screening

The ethanolic extracts of arils, albedo and peels were tested for the presence of: Total phenolic contents and Antioxidant scavenging activity. Ethanolic extract were determined according to the method of Mekni [18] for antioxidant scavenging activity and the method of Mutahar [22] for total phenolic contents. Results were expressed on a dry weight (DW) basis as mg gallic acid equivalents (GAE) /g of sample.

Antioxidant activity DPPH radical scavenging activity

Antioxidant activity of ethanolic arils, albedo or peels pomegranate extracts was evaluated through the measurement of the free-radical scavenging capacity by the DPPH· (2,2-diphenyl-1-picrylhydrazyl) assay [18]. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding with DPPH. The reduction (%) values were determined and compared to appropriate standards. Inhibition of the free radical DPPH is in per cent (I %).

Determination of total phenolic content

The total phenolic content of all extracts was determined using the Folin-Ciocalteu method described Mutahar [22]. Briefly, 0.5 ml of diluted extract was added to a test tube and then mixed with 5 ml of Folin-Ciocalteu reagent (0.2 N). After 8 min, 2 ml of Na_2CO_3 (15%) was added. The reaction mixture was incubated at 50 °C for 15 min before the absorbance (at 760 nm) of mixtures was recorded against a blank. Total phenolic content of the extracts was calculated from standard gallic acid solutions (0–0.1 mg/ml), and expressed as mg gallic acid equivalents (GAE) per 100 g fruit dry weight.

$$\frac{(50\ \text{g})\,(0.5\ \text{ml})\,(Y\ \text{g})}{(Z\ \text{g})\,(X\ \text{ml})}.$$

Microorganisms and culture

A total of 7food-related bacteria, yeast and fungi were kindly provided by the Culture Collection of Department of Microbiology, Faculty of Science, King Abdul-Aziz University of Saudi Arabia. They are two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-

negative bacteria (*Escherichia coli* and *Pseudomonas. aeruginosa*), yeast *(Candida albicans*) and fungi (*Aspergillus flaves* and *Aspergillus niger*). The strains were maintained at 4 °C in nutrient agar and potato dextrose agar slants for bacteria and fungi respectively. Before experimental use, cultures from solid media were sub-cultivated in broth medium, incubated for 24 h at 37 °C, and used as the inocula for the determination of antibacterial activity.

Determination of antimicrobial activity

The modified agar well-diffusion method Ahmad and Beg [1] was conducted to evaluate the antibacterial activities of the pomegranate parts extracts. A freshly grown culture was serially diluted, and 0.1 ml of prepared cells ($1.5x10^7$) colony forming units per milliliter, CFU/ml) was aseptically spread onto the surface of agar medium and then left to dry for 30 min. Wells (8 mm in diameter) were made in media using a sterilized stainless steel borer. Each well was filled with 30 µl of the ethanolic extracts. The plates were left at room temperature for 30 min to allow diffusion of materials in media. Plates were incubated at 37 °C for 18–24 h and 48 h for bacteria and fungi respectively. Inhibition zones in mm (including well diameter) around wells were measured. The antimicrobial activity was expressed as the diameter of inhibition zones produced by the extracts against test microorganisms [28; 24].

3. RESULTS AND DISCUSSION

In the present study the arils extract showed a good radical scavenging activity followed by peel, while the albedo showed the lowest mean antioxidant activity (Fig 1). Furthermore previously study explains that the pomegranate arils showed extreme antioxidant activity exhibited higher phenolic content [23]. A good DPPH scavenging activity of the pomegranate extracted from the peel was also studied by Singh [29]. The DPPH free radicals scavenging activity in different pomegranate part ethanol extracts exceeded high antioxidants, the most active arils (81%), followed by peel (66%) and albedo (49%). The arils showed maximum inhibition in the DPPH radical scavenging assay [22]. In another studies the peel exhibited maximum DPPH radical scavenging activity compared to the arils [21].

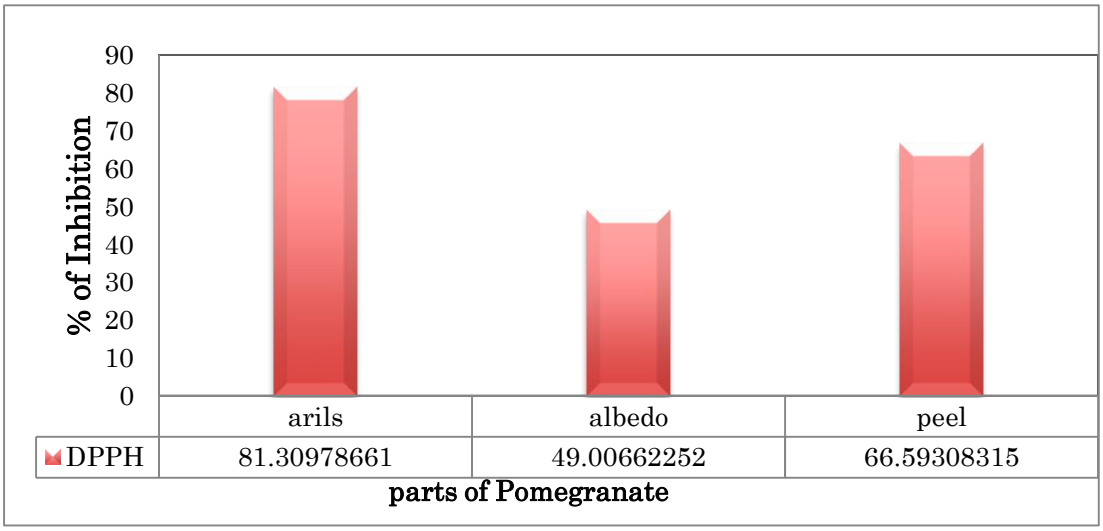

Fig 1: DPPH radical-scavenging activity of pomegranate

A likeness, research on the antioxidant activity of arils and peel of pomegranate demonstrated that the scavenging activity of all part of pomegranate on the DPPH radical was point to be strong. Over all, the distinction in the DPPH radical rummaging action of various concentrates incorporated that the separating dissolvable utilized would influence the radical searching power. This might be because of the diverse polarities of every cell reinforcement compound gathering present in pomegranate [8]. Proton gift causes the violet shaded DPPH radical swing to dreary non-radical compounds. In this radical capture activity could be tallied from DPPH radical scavenging. The remaining DPPH radical substance was spectrophotometrically estimated at λ 517 nm [13; 5]. The DPPH test was broadly utilized in characteristic item thinks about for cell reinforcement confinement and remove and unadulterated compound capacity to retain the radicals. Phenolic compounds are commonly found in pomegranate and have been reported to have several biological activity including antioxidant properties. Total phenolic content (TPC) of pomegranate of ethanolic arils, albedo and peel were reported in (Fig 2). The data showed that, the highest value of total phenolic content were appeared in arils pomegranate 42 mg/gdw, while the albedo and peel recorded the lowest total phenolic content 23 and 22 mg/gdw respectively. These outcomes proposed that diverse sorts and dimensions of phenolic compounds (e.g. flavonoids and tannins) are available in various parts of pomegranate each having an explicit antioxidant activity. The existence of phenolic compounds in arils is understood to be associated with their protective mechanisms during certain adverse conditions. Higher amount of phenolic compounds is produced stage of the growth this result is in agreement with Carballo [8] who studied the physical and chemical characteristics of 19 pomegranate cultivars and found that the amount of total phenolic varied between 354 and 783 mg/g in order to prevent the photooxidative damage and sea grazers, respectively. On the other hand there is a favorable correlation between the total phenolic and DPPH-scavenging activity signification of the summary reference that the presence of phenolic contents within the pomegranate may be the major factor to the antioxidant activity of pomegranate. Numerous studies have tested the function of phenolic contents in relationship of antioxidant activity [16]. The compounds have a place with phenolic, flavonoid, tanin, and alkaloid gatherings, and have numerous sulfide gatherings. What's more, a progression of polyphenolic compoundsand related phenolic compounds, for example, cathechol, caffeic corrosive, myricetin, epigallocathecin, and hespedirin have been secluded from similar tipe of pomegranate [2]. The mode of action of phenolic compound as a cancer prevention agent depends on the structure of sweet-smelling rings that joined to the hydroxyl groups [22].

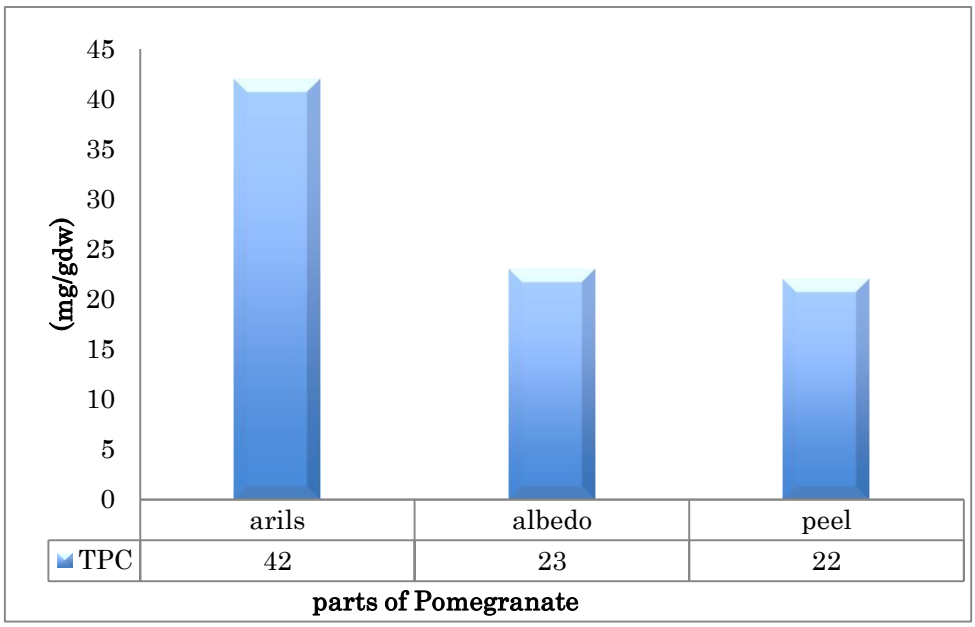

Fig 2: Total phenolic content (TPC) of pomegranate

The hydrogen particle of the hydroxyl gathering will be given to the temperamental free radicals and in this manner ending the oxidative action. In any case, negative connection between aggregate phenolic substance and cell reinforcement limit existed in a few investigations [4]. Later on, distinguishing proof of these atoms will be useful to comprehend the diverse cancer prevention agent components saw in these investigations. The antimicrobial effects of ethanolic extracts of different parts of pomegranate (arils, albedo and pee) on the growth of various Gram positive, negative bacteria and fungi using agar diffusion method are shown in Fig (3). The extracts display respectable antibacterial activity against Gram positive as well as Gram a negative bacterium that confirm previous study [30]. The pomegranate ethanolic extracts exhibit phenomenal degrees of antimicrobial activities against several microorganism, whereas peel have high active against all tested bacteria which was in agreement with other reports [19], while arils showed moderated activity against tested strains, but the albedo pomegranate found to be more resistant. In general, the most of tested pomegranate showed inhibitory activity against the tested bacteria (*E.coli, S. aureus* and *Bacillus subtilus*) Fig (4). The *Bacillus subtilus* and *Staphylococcus aureus* were found to be more sensitive (widest zones of inhibition) among the Gram negative bacteria (*E.coli* and *P. aeruginosa*), *C.albicans* and *A.niger A.flavus* was found to be more resistant as shown in Fig (3).

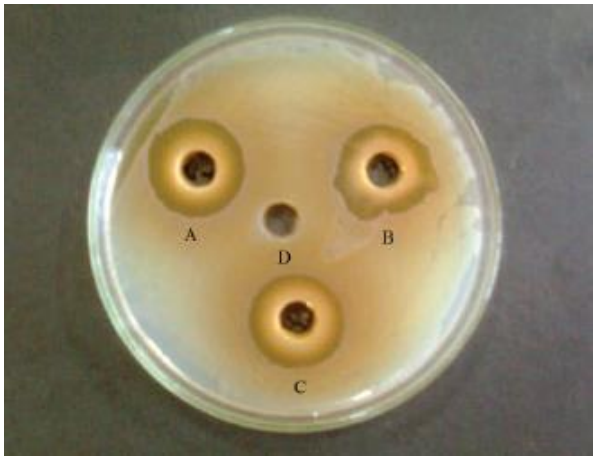

Fig (3): Agar plates containing zones of inhibition among the Gram positive bacteria *(Bacillus subtilus*), of ethanol extracts of pomegranate where (A) arils, (B) albedo (C) peel and (D) control

On the other hand, Gram negative bacteria were more resistant (without zones of inhibition), than the Gram positive bacteria. The Gram-positive bacteria were more sensitive to volatiles extract than the gram-negative bacteria, this observation is in concordance with a previous study conducted by Pagliarulo [25] who reported that are the Gram-positive bacteria were more sensitive to ethanol extracts of volatile compounds of Lamiacae. Overall, antibacterial activity of ethanolic extracts in (Fig4) showed pomegranate profoundly distinct antibacterial activity by having observable inhibition with diameters ranging from12 to 26 mm on tested bacteria. In this study, the peel and arils extracts were found more active than albedo extract. However peel extracts yield higher antibacterial activity than arils extracts which was in parallel with earlier investigation [9]. However, Mekni [18] reported that most of the active compounds of pomegranate showed antimicrobial activities. Brahmi [7] suggested that many metabolites isolated from pomegranate been shown to possess bioactive effects the antimicrobial activity of volatile compounds. Ahmad and Beg [1] recorded that the phytochemical substance found in ethanolic extract of pomegranate are phenol, alkaloid, glycoside, flavonoid, and tannin.

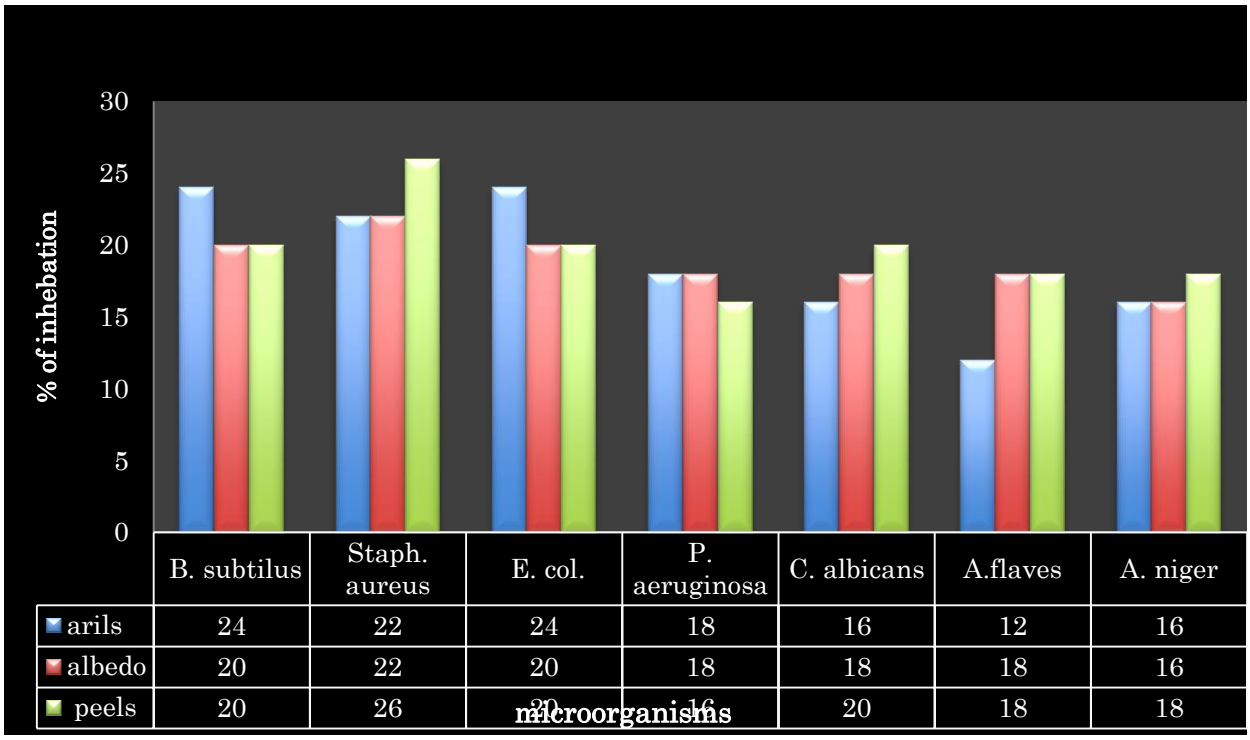

Fig 4: The diameter of inhibition zone (mm) surrounding pomegranate parts in presence of various microorganisms

Meanwhile, Antimicrobial activity be based on pomegranate species and on extraction efficiency as well as their active compounds location. In contrast, our results showed that the ethanol extract of pomegranate inhibited all the test organisms. This difference may be attributed to location or seasonal variations [17].

4. CONCLUSION

Other than having high phenolic substance and antioxidant powerful, pomegranate arils additionally have antibacterial action and might be utilized as prescription for people. This lessens the expense and the danger of anti-toxin utilization. Besides, included an incentive from the antibiotic which is the result could give medical advantages to people and might be utilized in sustenance protection and pharmaceutical purposes.

CONFLICT OF INTEREST

No conflict of interest

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