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Research Article

The Phytochemical and Antimicrobial Effect of *Mallus domestica* (Apple) Dried Peel Powder Extracts on Some Animal Pathogens as Eco-Friendly

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ABSTRACT

A noticeable potency of many species of plants present against bacterial and fungal pathogens. Bacterial resistance against antibiotics is consider one of the common problems in the medical world, so one of the most important steps in microbiological researches is to find a new antimicrobial compound with minimal side effects .So the aim of this study is to investigate the antimicrobial activity of *Malus domestica* (apple) peel aqueous and organic solvant extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration. Hot and cold aqueous in addition to ethanol extracts of *Malus domestica peel* were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerogene, Bacillus cereus and Candida albicans)* by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples. Also, phytochemical compoud of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Malus domestica* contain glycosides and flavnoids, tannins compounds while alkaloids and saponin not found. So, it could be concluded that the Malus domestica extracts possess remarkable antibacterial activity against gram-positive and gram-negative bacteria in addition to its antifungal activity against *Candida albicans and* to be introduced as an alternative to chemical antimicrobial drugs, is required wider investigation also it is considered as ecofriendly can be recycled and used as food additive in animal ration.

Key words: Apple, Antimicrobial, Extract, *Malus domestica*, Fruits peel

INTRODUCTION

The main food source for some essential nutrients is fruits and vegetables and also includes a series of bioactive components, which might have multiple effects in the fields of health (Sara *et al*., 2013) and (Yigit *et al*., 2009). Fresh, in juices, and cider fruits are different forms of fruit consumption including apples.

The apples high capacities in phenolic contents have several useful aspects for everybody's health (Sara *et al*., 2013) and (Alberto *et al*., 2006). various biological functions such as astringent, antioxidant, anticancer, anti-inflammation, and antibacterial activity that have been possessed due to Phenolic compounds include a significant class of phytochemicals (Sara *et al*., 2013) (Jeong *et al*., 2009) (Rubnov *et al*., 2001) (Ryu *et al*., 1998) and (Vaya and Mahmood, 2006). Among others, the apple polyphenols antimicrobial properties of have been extensively examined (Sara *et al*., 2013) (Ju and Bramlage, 1999) (Lu and Foo, 2000) and (Robards *et al*., 1999).

In addition, incidence or survival/growth of *Listeria monocytogenes*, *Listeria innocua*, *Salmonella serovars*, and *Escherichia coli* O157:H7 in fruit juices and apple cider has been demonstrated (Sara *et al*., 2013) (Raybaudi *et al*., 2006) (Raybaudi *et al*., 2009) (Ceylan *et al*., 2004) (Harris *et al*., 2003) (Ingham *et al*., 2006) and (Miller and Kaspar, 1994).

All over the world Malus domestica (apple) is a kind of fruit that is consumed widely (Sara *et al*., 2013) and (Shoji *et al*., 2004). Small and deciduous, reaching 3 to 12 metres (9.8 to 39 ft) tall, with a spreading canopy Size and shape of the plant is mainly depends on rootstock and training system (Vaibhav *et al*., 2012).

More over traditional herbal therapy can be a satisfying option where some of the pathological conditions where the scientific drug become crippled but demands an ample amount of research (Gupta *et al*., 2006).

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So, the aim of this study is to investigate the antimicrobial activity of *Malus domestica* (apple) peel aqueous and organic solvant extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration.

MATERIALS AND METHODS

Collection of plant materials: Fresh *Malus domestica* (apple) bright in color with no bruises were purchased from a local market. The apple was washed under running water and wiped with clean cloth to dry before peeling. The peels were removed using a sterilized sharp knife. On an average around 200 g of peel was obtained from 1 Kg of each.

Aqueous extraction: Distilled water was boiled, dried plant peel was added to the water and left to cool. Then were mixed by the blender and filtered to get clear aqueous extracts. The extracts were kept at 4°C until to be use. But the hot aqueous extract has been prepared directly after boiling and filtration according to (Rehab, 2017).

Solvent extract: 5 g of dried plant peel powder was extracted with 10 ml of each solvent (Ethanol) kept for 24 h. Then, it was filtered using Whatman No.1 filter paper. The solvent was evaporated to make the final volume as 1/2 of the original according to (Rehab, 2015).

Preparation of inoculums: The inoculums (bacterial strains and fungi) were isolated from large animals and poultry farms on the outskirts of Cairo. The strains of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerogenes, Bacillus cereus)* & fungi (*C. albicans)* were inoculated Sabaroud dextrose agar (SAB) (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) & nutrient broth (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) for overnight at 37°C for bacteria & 25°C for fungi according to (Rehab, 2017).

Antimicrobial screening: The agar well diffusion method was used for the determination of antibacterial activity of *Malus domestica* (apple)peel aqueous extracts in addition to ethanol extract by using bacterial isolates taken from animals and poultry (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerogenes, Bacillus cereus)* & fungi (*C. albicans)*to evaluate its effects on the isolated bacteria. Loopfull growth from bacterial isolate was inoculated into nutrient broth incubated at 37 °C for 18 hours. The dilution of bacterial suspensions with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to get a uniform suspension containing 1.5×108 CFU / ml. Muller- Hinton agar was inoculated with 0.1ml of bacterial inoculums. Using cork borer, wells were made on the cultured media. The aqueous and solvent extracts were considered as the 50% concentration. Then, 0.1ml of extracts were added to wells, then the plates left for 30 min in refrigerator at 4°C, thereafter, they were incubated at 37°C for 24 hrs. The activity of extracts was determined by measuring the diameter of inhibition zone in millimeter. All experiments were duplicated. Ciprofloxacin (10 µ g) and penicillin (10 µ g) used as positive control while distilled water (100 µg) used as negative control for antibacterial screening aqueous extract. Nystatin (10 µg) was used as positive control while distilled water (100 µg) used as negative control for antifungal screening. All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) (Rehab, 2017) and (Kanife, 2011).

Phytochemical tests:

Tannins Test: A modified methods stated in (Rehab, 2017) and (Kanife, 2011) was used to be presented of tannins on the extracts, A few drops of Ferric chloride reagent were added for 3 ml of extract. A blue black color refereed to the present of tannins.

Alkaloids test: A few drops of Marqus reagent (prepared from mixing 0.5 ml of Formaldehyde with 5ml of concentration H2SO4), added to the 5 ml of extract. Turbidity refereed to the present of alkaloids (Rehab, 2017) and (Ajoku *et al*., 2015).

Saponins test: 3 ml of extractwas added to the 2 ml of Fe rric chloride, a white residue to be formed as evidence to the present of Saponins (Rehab, 2017) and (Doss *et al*., 2011).

Flavonoids test: Flavonoids test were implement in conformity with (Rehab, 2017) and (Doss *et al*., 2011). 2ml of extract mix with Alcoholic KOH (0.5 mol.), a yellow color as proofed to the present of Flavonoids.

Glygosides test: 0.5g of grinded *Malus domestica* (apple) dried peel was dissolved in 2ml of glacial acetic acid containing one drop of Ferric chloride solution, and then under laid with 1 ml of concentration H2SO4A brown ring indicated the present of Glycosides (Rehab, 2017) and (Antia *et al*., 2010). All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company).

RESULTS

The results given in (Table 1) indicate that the hot and cold aqueous in addition to ethanol extracts of *Malus domestica dried peel* were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerogenes*, *Bacillus cereus)* & fungi (*C. albicans)* by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples. Also, phytochemical compoud of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Malus domestica peel* contain glycosides and flavnoids , tannins compounds while alkaloids and saponin not found as shown in (Table 2).

Table 1: Antimicrobial activity of *Malus domestica* (apple) peel aqueous and ethanol extracts against some animal pathogen in (mm)

Examined fruits and vegetables peels powder	Types of extracts	Types of examined microbes				
		S aures	Bacillus	*E coli*	Ps. eurogenes	*C albicans*
Malus domestica	Hot aqueous extract	30	27	21	15	20
	Cold aqueous extract	0	0	29	21	16
	ethanol	0	25	16	11	12
Control +ve	Ciprofluxacin	25	34	20	30	0
Control +ve	Bacitracin	33	36	17	22	0
Control +ve	Nystatin	0	0	0	0	16
Control -ve	Distelled water	0	0	0	0	0

Table 2: The phytochemical compounds in *Malus domestica* (apple) peel aqueous extracts

Plant extracts → / Phytochemical tests ↓	*Malus domestica*
Flavonoids test	+
Alkaloids test	-
Glygosides test	+
Saponins test	-
Tannins test	+

+ (contain this phytochemical compound).

DISCUSSION

From the ancient time different cultures around the world have used herbs and plants as a remedy in different diseased condition and maintain health. Many drugs prescribed today in modern medicinal system are derived from plants. Synthetic drug is known for its toxicity which sometimes needs serious medical attention. So, in the recent past the practice of herbalism has got popularity around the globe including the developed countries due to its potency and apparent safety profile. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems (Vaibhav *et al*., 2012) and (Adewusi and Afolayan, 2010). More over some of the pathological condition where the scientific drugs become crippled but traditional herbal therapy can be a satisfying option which demands an ample amount of research (Vaibhav *et al*., 2012) and (Gupta *et al*., 2006).

Peels of various fruits and vegetables are generally considered as waste product and are normally thrown away by us. But different studies conducted on peels revealed the presence of important constituents, which can be used for pharmaceutical purpose. Number of components having activities like antioxidant, antimicrobial, anti inflammatory, antiproliferative etc. have been isolated from different peels. (Sonia *et al*.,2014). The present study aimed to investigate the antimicrobial activity of Malus domestica (apple) peel aqueous and organic solvant extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration.

Effect of water and alcohol extracts of M.domestica fruit was found to be most effective against gram +ve and gram –ve bacteria such as B.subtilis, S.aureus and E.coli, P.aeuroginosa respectively (Vaibhav *et al*., 2012) and (Sun *et al*., 2002).

In agreement with the previous results as shown in (Table 1) the results indicate that the hot and cold aqueous in addition to ethanol extracts of *Malus domestica dried peel* were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenes, Bacillus cereus)* & fungi (*C. albicans)* by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples. It was clear that the hot aqueous extract most effective against gram positive bacteria and fungi where the cold aqueous extract most effective against gram negative bacteria,

In general, the Gram-positive strains of bacteria tested appeared to be more sensitive to the extracts. However, this study also records a significant susceptibility of some of the examined Gram-negative bacteria. According to literature, the antimicrobial activity could be influenced by the phenolic compounds (Sara *et al*., 2013) (Vaibhav *et al*., 2012) (Adewusi and Afolayan, 2010) and (Gupta *et al*., 2006).

fruits, as rich sources of phenolic compounds have been paid a special attention (Sara *et al*., 2013) (Robards *et al*., 1999) (Kalt *et al*.,1999) and (Wang and Lin,2000).Apple fruit contain several health and sensory related constituents including dietary fibre, sugars, vitamins and phenolic compounds (Sara *et al*., 2013) and (Hagen *et al*.,2007).The antioxidant capacity of apple is mostly attributed to phenolic compounds such as flavonoids and phenolic acids (Sara *et al*., 2013) (Eberhardt *et al*., 2000) and (Lee *et al*., 2003).

Moreover, Alberto (2006) reported that there is a direct relationship between phenolic content and antibacterial effect in four apple cultivars (Sara *et al*., 2013) and (Alberto *et al*., 2006). and their polyphenol extracts had stronger inhibition effects on the bacteria. (Sara *et al*., 2013) and (Alberto *et al*., 2001). An *in vivo* assay is necessary to confirm the antimicrobial activities of *Malus domestica*, which could be usefully applied to the food, pharmaceuticals, and cosmetics industries. Isolation of the gene responsible for the antimicrobial activity would be an interesting future study topic aimed at identifying the molecule generating the desirable efficacy.

The phytochemical content and antioxidant activity of the peels of Rome Beauty, Idared, Cortland, and Golden Delicious were compared to those of the flesh and flesh-peel combination components and peels of the apples. The result showed total phenolic and flavonoid contents were quantitatively more in the peels, followed by the flesh-peel combination and then flesh. The high content of phenolic compounds and antioxidant activity of apple peels indicates that they are valuable source of

antioxidants and can impart health benefits when consumed (Sonia et al., 2014) and (Wolfe *et al*., 2003).

Considering the previous results phytochemical compoud of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Malus domestica peel* contain glycosides and flavnoids, tannins compounds while alkaloids and saponin not found as shown in (Table 2). These findings are in agreement with measured total phenolic contents of the samples (Sara *et al*., 2013) (Drogoudi *et al*., 2008) (Lata, 2007) (Tsao *et al*., 2005) (Vieira *et al*., 2009) (Alberto *et al*., 2002) (Alberto *et al*., 2004) and (Xiangyang, 2003).

Conclusion

One of the most an interesting example of a plant used in traditional medicine for many years was M. domestica which were supported and proved to be useful for clinical studies and development of commercial drugs by the reported phytochemical and pharmacological studies

The hazardous effects of synthetic antibiotic and the emergence of antibiotic resistant strains have revived the search for antioxidant and antimicrobial agents from natural sources. From different studies conducted on peels, it has been found that peels of fruits and vegetables hold a tremendous potential to serve as a source of newer, effective, safer and better antimicrobial agents.

The fruit and vegetable peels are considered to be novel, easily available, efficient, affordable, eco-friendly, natural and economic source for antioxidants and antimicrobial agents especially here as recommended for recycling the apple peel as animal fodder where food and treatment or to be added in ration as dried powder.

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