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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (6): 1057-1061, 2004
ISSN 1028-8880

Antimicrobial Activity of Atung (*Parinarium glaberrimum* Hassk) Fruit Extract

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Abstract: An experiment was carried out to evaluate the antimicrobial activity potential of organic extracts of atung (*Parinarium glaberrimum* Hassk) fruits. Dried ground powder peels and seeds of atung (*P. glaberrimum* Hassk) were extracted with hexane, ethylacetate and methanol. The antimicrobial activity of these extracts was evaluated against bacteria, fungus and yeast using paper disc assays. The seed extracts had stronger antimicrobial activity than the peel extracts. The seed ethylacetate extract exhibited strong antimicrobial activity against six strains of food spoilage and pathogenic bacteria, nine strains of fruits and vegetables postharvest diseases fungus and four pathogenic yeast strains. The seed hexane extract showed weak antimicrobial activity, while the methanol extract had none. Ideally food preservatives have to be able to inhibit the growth of fungi, yeast and bacteria and therefore it may be considered as potential antimicrobial agents for use in food products and postharvest diseases control.

Key words: Antimicrobial activity, *Parinarium glaberrimum* Hassk, postharvest diseases

INTRODUCTION

Food-borne illness resulting from the consumption of foods contaminated with pathogenic bacteria or their toxins have been of great concern in the public health. There is, however, a strong demand for a reduction or elimination the use of synthetic chemicals in postharvest fruits and vegetables. This opinion is based on the increased concern about the health risks associated with the use of the synthetic chemicals, especially in the children[1-3].

Some plants contain bioactive compounds such as glycosides, alcaloids and terpenes, which may be used as drugs and anti-microbial agents[4]. Many extracts and essential oils have been derived from plants which have bactericidal, fungicidal and insecticidal properties[5-6].

Parinarium glaberrimum Hassk, locally known as "atung" is classified into (Rosaceae) Family, (Parinarium) Genus and (*P. glaberrimum* Hassk) Species[7]. Atung usually grows in the tropical forest habitat, tropical zone especially in lowlands from 0-300 m above the sea level. It is found abundantly in the tropical forest of Molucan Islands, Indonesia. The fruit is an oval shape, consists of a brown hard peel of about 3-4 cm and hard seed[8]. In Ambon, Indonesia atung seed is used in the traditional preservation of fish, flavouring in pineapple salads and raw fish, treatment of diarrhoea and bleeding in pregnant women.

Atung seed contains peculiar chemical compounds that may be act as antimicrobial substances. The raw powder of atung seed has been m shown to increase the shelf-life of *Penaeus monodon* Fab shrimp from 6 to 17 h[8]. Therefore present study was conducted to find out the antimicrobial substances that may be contained in atung fruit. This study also present data on the antimicrobial activity of extracts from atung fruit. The efficacy of hexane, ethylacetate and methanol extract from atung fruit against six bacteria, nine fungus and four yeasts was investigated and described.

MATERIALS AND METHODS

Plant materials: Fruits of atung (*P. glaberrimum* Hassk) were collected from Ambon, Maluku Province, Indonesia (0-10°S and 120-130°E). The fruits were cut into two pieces vertically then separated into peels and seeds (Fig. 1.). These were air-dried, grated and ground to powder separately.

Extraction method: Three organic solvents such as hexane, ethylacetate and methanol were used to extract of antimicrobial compound from atung fruits. These solvents were chosen on the basis of their degree of polarity. Eight fifty four g of dried-powder seeds were extracted subsequently with 500 ml of hexane, ethylacetate and methanol. After three days, the extracts were filtered,

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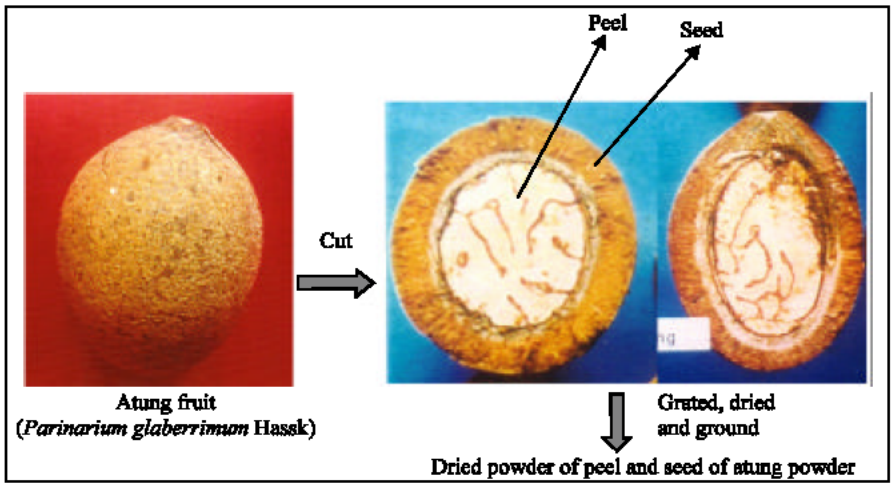

Fig. 1: Atung fruit (*Parinarium glaberrimum* Hassk)

concentrated and collected into *vacuo*. Extracts were re-suspended in methanol to achieve the stock concentration 2 mg ml^{-1}. The dried-powder peels were also treated in the same manner.

Microorganisms and culture media: The following microorganisms were used to assess the antimicrobial activity. 1. Gram positive bacteria : *Bacillus cereus*, *Bacillus subtilis* IFO-13719, *Micrococcus luteus* IFO-12708, *Staphylococcus aureus* IFO-14462. 2. Gram negative bacteria : *Escherichia coli* IFO-3301 and *Salmonella enteritidis* IFO-3313. 3. Fungi : *Rhizopus stolonifer*, *Colletotrichum acutatum*, *Geotrichum candidum*, *Penicillium expansum*, *Penicillium italicum*, *Fusarium oxysporum lycopersici*, *Botrytis cinerea*, *Phytophora citrophora* and *Monilinia fructicola*. 4. Yeast : *Candida albicans*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Zygosaccharomyces* sp. The fungi and yeast were obtained from The Gene Bank, Ministry of Agriculture, Forestry and Fisheries Tsukuba and the Plant Pathology Laboratory, Kagoshima University, Japan. The culture media for bacteria consisted of peptone (1%), meat extract (1%) and NaCl (0.5%) at pH 6.5-6.6. While yeast and fungi media was made up of malt extract (2%), glucose (2%) and peptone (1%), at pH 5-6.

Assay of antimicrobial activity using a paper disc assay: The antimicrobial activity was measured by the disc assay procedure. Discs were used as assay plates. Soft agar medium culture (3.5 ml) seeded with the tested microorganisms was layered over 10 ml of hard agar (2%). A 20 µl of each test extract was impregnated into paper discs (Toyo Roshi, Co., Japan) of 6 mm diameter and 1.5 mm thickness. After drying, the paper discs were placed on the assay plates. The agar was allowed to solidify before incubation at 30°C for 24 h for bacteria and 25°C for 4 days for fungi and 2 days for yeasts. The diameter of clear zones around each disc was measured after incubation to determine the strength of inhibition.

RESULTS AND DISCUSSION

From the dried powder of atung (*P. glaberrimum* Hassk) peels and seeds antimicrobial compound were extracted with hexane, ethylacetate and methanol by soxhlet extraction method. The seed ethylacetate extracts had the highest yields with a dark-brown color. The hexane peels and seeds extracts had lower yield than the ethylacetate and methanol extracts (Table 1). The hexane peels and seeds extracts were light green and oily-yellowish, respectively. This phenomenon occurred due to the fact that non-polar organic solvent (hexane) was used to separate lipids before continued with the other solvent consumption[9].

The ethylacetate peel extract exhibited antibacterial activity, while both the hexane and methanol peel extracts did not exhibit antibacterial activity. Ethylacetate seed extracts showed stronger antibacterial activity than the hexane seed extracts. Methanol seed extracts, on the other hand, did not exhibit antibacterial activity during the experiment (Table 2). Ethylacetate seed extracts

Table 1: Extract yield of atung fruit

Extract solvents	Extract colour	Extract yield (g)
Peel		
Hexane	Light green	1.5
Ethylacetate	Brown	2.0
Methanol	Green	2.4
Seed		
Hexane	Yellowish	18.7
Ethylacetate	Dark brown	80.2
Methanol	Blackish brown	18.7

Table 2: Antibacterial activity of atung fruit extracts against food spoilage and pathogenic bacteria

	Antibacterial activity*					
	Peel extract			Seed extract		
Test organism	Hexane extract	Ethylacetate extract	Methanol extract	Hexane extract	Ethylacetate extract	Methanol extract
Gram-positive						
Bacillus cereus	-	+	-	+	+++	-
Bacillus subtilis	-	+	-	+	+++	-
Staphylococcus aureus	-	+	-	+	+++	-
Micrococcus luteus	-	+	-	+	+++	-
Gram-negative						
Escherichia coli	-	+	-	+	+++	-
Salmonella enteritidis	-	+	-	+	+++	-

*Diameter inhibitory zones : –(negative) = 0 mm; + (positive) = 1–3 mm; ++ (moderate) = 4–6 mm and +++ (strong) ≥ 7 mm

Table 3: Antimicrobial activity of atung fruit extracts against yeast pathogen

	Antiyeast activity*					
	Peel extract			Seed extract		
Test organism	Hexane extract	Ethylacetate extract	Methanol extract	Hexane extract	Ethylacetate extract	Methanol extract
Candida albicans	-	-	-	++	+++	-
Pichia anomala	-	-	-	++	+++	-
Sacchromyces cerevisiae	-	-	-	++	+++	-
Zygosaccharomyces sp.	-	-	-	++	+++	-

*Diameter inhibitory zones : -(negative) = 0 mm; + (positive) = 1–3 mm; ++ (moderate) = 4–6 mm and +++ (strong) ≥ 7 mm

Table 4: Antifungal activity of atung fruit extracts against fungal postharvest diseases

	Antifungal activity*					
Test organism	Peel extract			Seed extract		
	Hexane extract	Ethylacetate extract	Methanol extract	Hexane extract	Ethylacetate extract	Methanol extract
Rhizopus stolonifer	-	-	-	+	+++	-
Colletotrichum acutatum	-	-	-	+	+++	-
Geotrichum candidum	-	-	-	+	+++	-
Penicillium expansum	-	-	-	++	+++	-
Penicillium italicum	-	-	-	++	+++	-
Botrytis cinerea	-	-	-	+	++	-
Phytophora citrophora	-	-	-	++	+++	-
Monilinia fructicola	-	-	-	++	+++	-
Fusarium oxysporum lycopersici	-	-	-	+	++	-

*Diameter inhibitory zones : -(negative) = 0 mm; + (positive) = 1–4 mm; ++ (moderate) = 5–8 mm and +++ (strong) ≥ 9 mm

effectively inhibited the gram positive and negative bacterial strains tested. The activity against both types of bacteria may be indicated the presence of broad spectrums antibiotic compounds or simply general metabolic toxin. These results in agree with that of the previous studies[10-12]. Many reports[13-16] show that spices, herbs and other plant materials possess antimicrobial activity. Ethylacetate seed extracts of had a strong activity against *E. coli* and this finding is related with the use of atung seed powder as a traditional diarrhoea medicine[17].

Antiyeast of atung fruit extracts is presented in Table 3. Peel extracts did not show antiyeast effects, but seed extracts did. The seed extracts of hexane and ethylacetate had anti-yeast activity against *Candida albicans*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Zigosaccharomyces* sp. However, ethylacetate seed extracts showed stronger antiyeast activity than hexane extract. It was reported that many plant extracts were effective against yeast like Kumazasa (*Sasa albo-marginata*) extract[10], cardamomum seed extract[18], aniseed seed extract (*Pimpinella anisum*)[19], cocklebur leaves extract[20] and pepperfruit extract (*Dennetia tripetala*)[21].

The antifungal activity of peel and seed extracts tested against nine fruits and vegetables postharvest fungal groups is presented in Table 4. Antifungal activity was not observed for the three peel extracts. The hexane seed extracts had a positive (+) to moderate (++) antifungal activity, while ethylacetate extract exhibited very strong positive (+++) antifungal activity. But the methanol seed extract did not show antifungal activity. A rapid screening has been developed for antimicrobial activity (natural compounds) against *Botrytis cinerea*[22]. It was found that essential oils derived from red thyme (*Thymus zygis*), cinnamon leaf (*Cinnamomum zeylanicum*) and clove buds (*Eugenia caryophyllum*) were the most effective among forty nines tested oils. Hinokitiol, derived

from the roots of Japanese Cypress (*Hiba aboraitae*), has been reported to extend the shelf-life of peaches[23] and control postharvest diseases in eggplant and pepper[24]. The ability of several essential oils in controlling decay in stored flower bulbs were reported, oil extracts from oregano (*Oreganum syriacum*) were found very effective[25].

In this study the stronger antimicrobial activity observed for the seed extracts over the peel extracts may be due to the fact that seed play an important role in storing chemical compounds produced by plants. Seeds contain cells that function in storing or accumulating certain active secondary metabolites. Some food spices like aniseed seed (*Pimpinella anisum* L)[19] and Cardamom (*Ellataria cardamomum* Zingiberaceae) seed show good effective antimicrobial activity[18]. Grape seed (*Vitis vinifera*) extract possess stronger antibacterial activity was reported[26]. In addition, it was reported that different part of plant has different active compounds[4]. In other study, it was found that an extract from bamboo bark possess stronger antibacterial activity than that from bamboo grass[27]. Different antibacterial activity among leaves, inner bark and outer bark of Marula (*Sclerocarya birrea*) was also found and the inner bark was the strongest one[28].

Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and protein, as well as the inhibition of the metabolism of nucleic acids[29]. It seems likely that substances in the extracts act separately or in concert to exert these effects. Taking into consideration the properties of the organic solvent used for the extraction, the extracts seem to contain diverse substances, ranging from non-polar to polar compounds.

In conclusion, the present study reports the ethylacetate extracts of atung seed had the most effective antimicrobial activity against all tested microbial. It produced substantial yields of biologically active ingredients as measured by the degree of inhibition and number of microorganisms inhibited. Ideally food preservatives have to be able to inhibit the growth of fungi, yeast and bacteria and therefore it may be considered as potential antimicrobial agents for use in food products and postharvest diseases control. Further experiments on the purification and isolation of the antimicrobial substances of ethylacetate extracts of atung seed are in progress.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Taing Ok for assistance with the paper disc method and Dr. Yuka Watanabe for her valuable criticisms with this manuscript. Thanks are also addressed to Dr. D.W. Widjajanto and Mr. Joseph for their valuable suggestions and assistance.

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