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Research article

Preliminary phytochemical screening of raw and thermally processed palmyra palm (*Borassus flabellifer* linn.) fruit pulp

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Abstract

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as Thailand, Malaysia, Indonesia, India, Myanmar, Sri Lanka and Cambodia. The palmyra palm (*Boraassus flabellifer* Linn.) fruit is one of the less known tropical fruits. The major reasons for the underutilization of these fruits are separation of the pulp from the fiber and its bitter taste. The fruits are stomachic, sedative, laxative and aphrodisiac in nature. The current study was conducted to screen the different phytochemicals including alkaloids, flavonoids, terpenes, glycosides, saponins, phenolics, tannin, carbohydrate, protein and steroids and sterols from aqueous and methanolic extracts of raw palmyrah palm fruit pulp (RPFP) and thermally processed palmyrah palm fruit pulp (PPFP). The result revealed that both the RPFP and PPFP contain all the phytochemicals except protein. The terpenes, glycosides, carbohydrate and steroids and sterols are present in appreciate amount and proteins are abscent in all the extraction of both samples. Alkaloids were present appreciably in the aqueous extraction of RPFP and present fewer amounts in PPFP. But methanol extraction of both RPFP and PPFP contain alkaloids strongly. The flavonoids, phenols and tannins were present in both the extraction of RPFP and PPFP. Glycosides were found in the aqueous extraction but present appreciable amount in methanol extraction of RPFP and PPFP. On processing alkaloids were reduced in the aqueous extraction of PPFP and saponin in both the extraction of RPFP and PPFP. Therefore no major changes in the presence of phytochemical components due to heat processing were found in this current study.

Key words: Raw palm fruit pulp (RPFP), processed palm fruit pulp (PPFP), aqueous extraction, methanol extraction, phytochemical screening.

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1. Introduction

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as Thailand, Malaysia, Indonesia, India, Myanmar, Sri Lanka and Cambodia [1].

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The palmyra palm is known simply as palmyra, which is based on the Portuguese palmeira, the tree was named originally for the resemblance of the leaf to the palm of a hand. Other names are toddy palm, wine palm, Cambodian palm and botanically known as *Borassus flabellifer* L. *Borassus* is from a Greek word describing the leathery covering of the fruit, Flabellifer means "fan-bearer" [2]. The palm belongs to the family *Arecaceae*, subfamily *Borassoideae* and genus *Borassus*. The palmyrah palms are slow-growing dioecious perennials and have no distinguishing features to identify the sex until flowering. The palm commences flowering only after 12–15 years of maturity. Inflorescences are interfoliar; the male inflorescence has stout terete branches, while the female inflorescence is more sparingly branched. Fruits are semi-globose to globose and deep brown to black when ripe [3]. The palmyra palm (*Boraassus flabellifer* Linn.) fruit is one of the less known tropical fruits. The coconut-like fruits are three-sided when young, becoming rounded or more or less oval, 12-15 cm wide and capped at the base with overlapping sepals. The outer covering is smooth, thin, leathery and brown, turning nearly black after harvest. Inside is a juicy mass of long, tough, coarse, white fibers coated with yellow or orange pulp. The pulp of mature fruits is sucked directly from the wiry fibers of roasted, peeled fruits. It is also extracted to prepare a product called punatoo in Ceylon. It is eaten alone or with the starch from the palmyra seedlings. The fresh pulp is reportedly rich in vitamins A and C [4]. Jayaratnam (1986) isolated tetraglycoside of the steroid and later the bitter compound called flabelliferrins, which are steroidal saponins and this bitter principle in *Borassus* was identified by Jansz *et al.* (1994) [5,6]. The major reasons for the underutilization of these fruits are separation of the pulp from the fiber and its bitter taste. Debittering is an important process for the utilization of the PFP. Heating the palmyra palm fruit over hot coals traditionally or using heat stable enzymes scientifically is reduced the bitterness [6,7].

The different parts of the plant are used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement [8]. The palmyrah plant has been used traditionally as a stimulant, anti-leprotic, diuretic, antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature. The roots and juice of the plant are useful in inflammatory reactions [9,10,11].

Phytochemicals are the bioactive, non-nutrient, and naturally occurring plant compounds found in fruits, vegetables, and whole grains. They can be categorized into various groups, i.e., polyphenols, organo-sulfur compounds, carotenoids, alkaloids, and nitrogen-containing compounds [12]. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [13]. There is the potential use of these tropical fruit pulps and their by-products to isolate specific phytochemicals for application in nutraceutical supplements, dietary additives, new food and pharmaceutical products, contributing to the recovery of agro-industrial process waste, with major industrial, economic and environmental impact. Therefore, the identification and

quantification of phytochemicals in the pulps and by-products of tropical fruits are of utmost importance to substantiate their potential health benefits in human nutrition [14].

Hence the present study was aimed at qualitative phytochemical screening from the raw palmyra palm fruit pulp (RPFP) and processed palmyra palm fruit pulp (PPFP) for further quantification of bioactive components.

2. Materials and method

Sample collection

The fully ripened palm fruits (50 samples) with distinct flavors and dispersed easily from the crown were randomly picked from the tree with the help of palm tree climber.

Pulp Extraction

The ripe palmyra fruits were thoroughly washed, the outer black skin was peeled off; fibrous part with fruit pulp were separated from the seed and extracted by home scale juicer. One part of the fresh pulp was filled in sterilized glass bottles and stored in the refrigerator and the rest were heated at 90˚C for 15 minutes, potassium meta bisulphate (0.14 g/Kg of pulp) was added as a preservative packed into sterilized glass bottles and finally the filled bottles were pasteurized (80˚C for 20 min) for further use.

Aqueous extract

The aqueous extraction was done by the cold maceration method described by Anowi (2012) with slight modifications in palmyra palm fruit pulp samples (RPFP and PPFP) separately [15]. 20 g of fresh fruit pulp was added to 200ml of water and allowed to stand for twenty four (24) hours. It was made into slurry by blending with another 100ml of water and then filtered through Whatman no. 1 filter paper and the extract was directly used for qualitative tests.

Methanol extraction

The alcoholic extraction method according to Cowan (1999) was used for methanol extraction of palmyra palm fruit pulp samples (RPFP and PPFP) separately [16]. About 20 g of the prepared palmyra palm fruit pulp samples were soaked in200ml of methanol for extended periods (about 24 hours). The slurry was then filtered through Whatman no. 1 filter paper and washed with 50ml of methanol, after which it was used directly for the qualitative phytochemical screening.

Qualitative analysis of Phytochemical screening

The following the methods of Horbone, (1973) and Trease and Evans (1989) were used in the phytochemcial screening of aqueous and methanol extraction of palmyra palm fruit pulp (RPFP and PPFP) [17,18].

Test for Alkaloids

Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagent were added to the mixture. Appearance of cream color precipitates with Mayer's reagent and appearance of reddish brown precipitates with Wagner's reagent indicates the presence of alkaloids.

Test for Flavonoids

Crude extract was mixed with 5ml of dilute ammonia followed by the addition of concentrated sulphuric acid. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow coloration disappears on standing.

Test for Terpenoids (Salkowski test)
Crude extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added sideways. A reddish brown coloration at the interface indicates the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani test)
Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of cardiac glycosides.

Test for Saponins
Crude extract was mixed with 5ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols
Crude extract was mixed with a few drops of 10 % solution of lead acetate. White precipitate indicates the presence of phenols.

Test for Tannins
Crude extract was mixed with 2 ml of neutral $FeCl_3$. A dark green coloration indicates the presence of tannins.

Test for Carbohydrates
Crude extract was mixed with a few drops of α naphthol solution in alcohol and concentrated sulphuric acid was added from the side of the test tube. Violet ring formed at the junction of two liquids showed the presence of carbohydrate.

Test for Proteins
Crude extract was mixed with 2 ml of Biuret reagent. The violet color indicates the presence of proteins.

Test of Steroids and sterols
The extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

Results and Discussion
The aqueous and methanolic extracts of RPFP and PPFP were screened for different phytochemicals including alkaloids, flavonoids, terpenes, glycosides, saponins, phenolic, tannin, carbohydrate, protein and steroids and sterols. The results of both aqueous and methanol extraction for phytochemical tests (Table 1) indicated that the RPFP and PPFP contain alkaloids, flavonoids, saponins, phenolics, tannins appreciably. The strong presence of alkaloids, flavanoids, phenols and tannins were evidence by the formation of strong colour and precipitate. The presence of terpenes, glycosides, carbohydrate and steroids, sterols and absence of proteins were also noticed in all the extraction of both samples. Alkaloids were present appreciably in the aqueous extraction of RPFP and present fewer amounts in PPFP. But methanol extraction of both RPFP and PPFP contain alkaloids strongly. The flavonoids, phenols and tannins were present in both the extraction of RPFP and PPFP. Glycosides were found in the aqueous extraction, but present appreciable amount in methanol extraction of RPFP and PPFP. The ethanolic, chloroform and aqueous extracts of fresh palmyrah fruit

pulp and sun dried fruit pulp (Pannatu) were studied and the extracts contain steroids, triterpenoids, carbohydrates, saponin, flavonoids and proteins in varied amounts in the ethonolic and water extracts which is similar to this current study except for protein. The chloroform extract showed negative results for all tested compounds except for carbohydrate in the same [19]. Glycosides, alkaloids and tannins were not observed in any of the extracts but all these were present in the both aqueous and methanolic extract of PFP samples in the current study. Another study showed that the ethanolic extract of PFP powder indicated the presence of saponins, flavonoids, glycosides and phenolic compounds [20]. In contrary, the alkaloids and tannins were absent in the same. The differences might be due to the varietal changes of fruit and different solvents used for the extraction.

On processing alkaloids were reduced only in the aqueous extraction of PPFP. The saponin was also decreased in both aqueous and methanolic extraction of RPFP and PPFP. The level of phytochemicals in vegetable and fruit processing decreases exponentially with a linear increase in blanching and boiling time [21]. Processing of fruit or vegetables can result in a significant reduction in phytochemical content. There are no changes in the presence of flavanoids, phenols and tannin in the raw PFP (RPFP) after thermal processing (PPFP). The results from phytochemical screening of bitter garden egg (*Solanum incanum*) fruit indicated that there is no much difference in raw and heat processed samples for 15 minutes [22] Glycosides are strongly present in the methanolic extraction than aqueous extraction which indicates the different solvents of different polarity is used to separate compounds based on their solubility in the extraction solvent [23].

Alkaloids are one of the largest groups of phytochemicals that have led to the invention of powerful pain killer medications [24]. They are also involved in protective function in animals and are used as medicine, especially the steroidal alkaloids [25]. Steroids are known to be an important Cardio tonic activities posse's antimicrobial property and also used in herbal medicines and cosmetics. The presences of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na^+ and K^+ pump that increases the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. Thus are used in the treatment of congestive heart failure and cardiac arrhythmia [26]. Flavanoids and tannin are the group of phenolic compounds that act as primary antioxidants and posse's antimicrobial, anti-inflammatory, anti-allergic, anticancer, anti-neoplastic activity, and for the treatment of intestinal disorders [27]. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea [28] Saponins which act as bioactive antibacterial agents in plants are also used to treat hypercholesterolemia, hyperglycemia and obesity [29]. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides [28]. Fruits that have tannins as their major components are astringent in nature. They are used in treating intestinal disorders such as diarrhea and dysentery as in [30, 31].

Table 1. Phytochemical screening of aqueous and methanol extract of RPFP and PPFP

Phytochemicals	Aqueous Extraction		Methanol Extraction	
	RPFP	**PPFP**	**RPFP**	**PPFP**
Alkaloids	++	+	++	++
Flavonoids	++	++	++	++
Terpenoids	+	+	+	+
Cardiac Glycosides	+	+	++	++
Saponins	++	+	++	+
Phenolics	++	++	++	++
Tannin	++	++	++	++
Carbohydrate	+	+	+	+
Protein	-	-	-	-
Steroids and Sterols	+	+	+	+

++ indicates strong presence, + indicates presence - indicates absence

Conclusion

The preliminary phytochemical screening is the fundamental step for quantitative estimation of bioactive chemical constituents present in the fruit pulp. There was no major alteration after thermal processing in the qualitative phytochemical compounds of the palmyra palm fruit pulp. Thus the phytochemical compounds present in the raw and processed palmyra palm fruit pulp can be extracted for the further drug development.

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