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Physicochemical Characterization of Pectin Extracted From Six Wild Edible Fruits In Odisha, India

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Abstract

The life of plant species flourishing in Odisha is luxuriant which is created based on its tropical climate type and assembles a remarkable extensive diversity in edible fruits. These wild edible fruits are predominantly utilized in tribal and rural livelihoods inhabiting in nearby forest regions. Despite their rich source of macronutrients, micronutrients, vitamins and antioxidants, they are often underutilized. Pectins are considered as food additives due to its natural dietary fibre consisting high proportion of galacturonic acid. The main aim of this study was to explore the pectin potential in these six wild edible fruits which might provide alternative commercialised products, value addition and favouring food security. Analysis of pectin yield and degree of esterification are considered as two important factors for marketable pectins. Equivalent weight is directly proportional to the formation of the gelling effect. In this study, highest pectin yield was recorded in *Citrus medica* (25.34 ± 0.77 % dry wt.) followed by *Phyllanthus emblica* (14.86 ± 0.40 % dry wt.) and *Carissa carandas* (11.94 ± 0.60 % dry wt.). To evaluate pectin suitability in food systems, characterization of extracted pectin was carried out following several parameters. The physico-chemical properties from dried pectin revealed highest degree of esterification (87.63 ± 1.33 %), equivalent weight (1516.66 ± 187.63), anhydrouronic acid (94.23± 2.67 %) and methoxyl content (14.62 ± 0.27 %) in *Phyllanthus emblica* whereas more moisture content of pectin was available in *Citrus medica* (10.35 ± 0.35 %). Our findings revealed that the methoxyl value and degree of esterification (DE) are classified as high methoxyl pectins (HMP), suitable for industrial and therapeutical applications.

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Keywords

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Introduction

Odisha indeed, is bestowed with a rich extensive diversity of wild edible fruits (WEF). Odisha's forests accommodate various wild relatives that are underutilized.[1] Since ancient, WEF played a major role in diet supplementation possessing nutraceutical potential (Table1.). Pectins are natural polymers, exceptionally complex polysaccharides which act as a thickening and stabilizing agent helpful in various food and pharmaceutical industries.[2] In terms of nutrition and health, pectin showed enormous therapeutic ability. AUA (Anhydrouronic acid) and methoxyl values help in determining the gel formation properties and purity of pectin respectively while the effect of gelling is based on its equivalent weight.[3] The DE is categorized for marketable pectins based on HMP and LMP (Low methoxyl pectin). Pectin obtained from *Citrus* fruit peel acts as a natural binder in paracetamol tablets.[4] *Carissa carandas* and *Sonneratia apetala* fruit species have been reported to be used for the preparation of syrup, jam, and sweet snacks. Till date, many cultivated fruits like peach, Banana, papaya, lemon, orange, sweet lime, apple pomace, grapefruit, guava, mango, pineapple, watermelon and avocado had been studied for its pectin value while reports on wild fruits are scanty.[5,6] Keeping in mind the growing need for alternative bionutrition resources, this piece of work aims to extract and characterize the physicochemical properties of pectin from these wild edible fruits and observe its potency for further commercialization and domestication.

a. *Carissa carandas* **b. *Citrus medica*** **c. *Ficus auriculata***

d. *Limonia acidissima* **e. *Phyllanthus emblica*** **f. *Sonneratia caseolaris***

Fig.1: (a-f): Six wild edible fruits of Odisha

Table 1: Phenology and medicinal uses of 6 wild edible fruits of Odisha

Sl. No.	Name of the wild fruit species	Local name (Odia)	Family	Fruiting season	Fruits medicinal uses
1.	*Carissa carandas* Linn.	Karanda koli	Apocynaceae	Jul- Oct	Used as an anticancer, antidiabetic, anthelmintic and adaptogenic activities.[7-10]
2.	*Citrus medica* Linn.	Limbu	Rutaceae	Oct- Dec	It acts as an analgesic, good antioxidant, anticancer and antiulcer properties.[11-14]
3.	*Ficus auriculata* Lour.	Raja dimiri	Moraceae	Oct- Dec	It possesses rich antioxidants, antimicrobial and antifungal activities.[15]
4.	*Limonia acidissima* Linn.	Kaitha	Rutaceae	Nov- Jan	It acts as an antidiabetic, anticancer, biosorbent wound healing and hepato-protective properties.[16-19]
5.	*Phyllanthus emblica* Linn.	Aonla	Euphorbiaceae	Nov- Jan	Ripe fruits are astringent, unripe fruit is laxative, antipyretic, analgesic and diuretic.[20, 21]
6.	*Sonneratia caseolaris* L.	Orua	Sonneratiaceae	Aug	Used as vegetables, acts as anthelmintic, relieves cough, antispetic.[22, 23]

Materials and Methods

Specimen Collection and Processing

Fresh mature fruits were collected from various forest regions like Kapurmal (Kalahandi Dist.); Kalimela (Malkangiri Dist.); Muktaposi, Khamara (Dhenkanal Dist.); Chandaka (Khurda Dist.); Nayagarh Dist.; Taptapani (Ganjam Dist.) and Bhitarkanika (Kendrapada Dist.) belonging to Odisha state, India(Fig.1). Immediately after collection, fruits were brought to the laboratory and cleaned thoroughly with tap water and oven dried at 50°C. After drying, the fruits were pulverized into powder form and in airless containers it was stored for further analysis.

Methodology for Pectin Extraction

The pectin extraction was carried out following Liew *et al.,*[24] with minor modifications. The fruit powder (10g) was weighed and mixed with distilled water (250 ml). The pH was set to 3.0 using 0.1 N citric acid. For blending, the sample mixture was stirred properly. The acidified samples were treated at 80°C for 75 min in a shaking water bath. After cooling, the solution was incubated for 24 h at room temperature. The obtained precipitated pectin was centrifuged for 10 min at 6000 rpm. The clear supernatant samples were filtered and ethyl alcohol (95%) was added twice the volume for pectin precipitation. The floatation of pectin was allowed by storing for 24 h in dark condition at 25°C. Using 70 % ethyl alcohol the resulted pectin was washed thoroughly and filtered. Drop wise acetone was added for removal of unwanted pectin colour.[25] The pectin obtained was oven dried at 50°C until an achievement of constant weight. The pectin yield in selected fruits was calculated as % dry wt.

Pectin yield(%):[pectin obatained(gm)÷Wt. of sample (gm)]×100 ...(1)

Degree of Esterification (DE)

The determination of DE had been carried out following Food Chemical Codex method.[26] Dried pectin (0.2g) was dispersed with ethyl alcohol and then dissolved in 20ml dist. H_2O. The sample solution was placed in an automatic shaking water bath at 45°C to dissolve completely. After cooling, phenolphthalein indicator (3 drops) was added into

the solution mixture and titrated against 0.1N NaOH till visibility of the pink colour. It is recorded as an initial titration volume. Further, 0.1N NaOH (10ml) was mixed and vigorously shaken for neutralization of polygalacturonic acid. It was placed at room temperature for 2 h. 0.1N HCl (10ml) was added to the sample mixture and shaken until the disappearance of pink colour. Then phenolphthalein indicator (3 drops) was mixed to the solution and further titrated using 0.1N NaOH and the recorded once the pink colour is seen which is considered as the final titration volume. The Degree of esterification was calculated using the formula given below:

D.E.(%):[{Final vol.}÷{Initial vol.(ml)+Final vol. (ml)}]×100 ...(2)

Equivalent Weight

The equivalent weight has been analyzed using Ranganna.[27] Pectin sample (0.1 g) was weighed. Then, it was blended with ethyl alcohol (5ml) and Sodium chloride (1.0g) where the volume was made up to 100ml using distilled water. Titration procedure was carried out using 0.1 N NaOH after adding a phenol red indicator (6 drops). The pink colour obtained was considered as the end point. Using the formula given below, the equivalent weight was determined.

Equivalent Weight (Eq Wt.):[{Wt.of pectin sample (gm)×1000}÷{initial vol.(ml)}N] ...(3)

Where, N= Normality of alkali

Methoxyl Content

Methoxyl content has been performed following the Ranganna method.[27] The neutral solution obtained for equivalent weight determination was taken where, 0.1N NaOH was added and the mixture was stirred vigorously. Then it was incubated for 30 min at room temperature. Further, the solution mixture was titrated after adding 0.1N HCl (25ml) with 0.1N NaOH. The calculation of methoxyl content has been executed with the equation given below:

Methoxyl content (%):[obtained alkali (ml)×N×3.1]÷sample wt. ...(4)

Where, N= Normality of alkali

Moisture Content

The determination of moisture was examined following AOAC method.[28] A dried empty petri dish was taken where the pectin powder sample (0.5gm) was weighed and transferred into it. It was placed in oven at 50°C for 3hrs. Then the petri dish was cooled down in a desiccator and the remaining final residue was recorded. The moisture content was calculated as mentioned below:

Moisture (%):[Weight of the final residue (gm)÷initial wt.of sample (gm)]×100 ...(5)

Anhydrouronic Acid Content (AUA)

The sugars, galactose and arabinose constitute polygalacturonic acid which is esterified partly. Using known amount of methoxyl content and equivalent weight of pectin, AUA was calculated using the given formula:

AUA(%):[(176×0.1Z×100)/(W×1000)+(176×0.1Y×100)/(W×1000)] ...(6)

Where, Z: vol. (ml) of sodium hydroxide obtained from equivalent weight

Y: vol. (ml) of sodium hydroxide obtained from methoxyl content

W: sample weight (gm)

Statistical Analysis

The results were demonstrated with mean and standard error (dry wt. basis) of triplicate observations. The statistical data was appraised by one-way ANOVA using Graph Pad Prism 8.0 software where the values of all selected parameters were differentiated significantly (P- value - 0.05) regarding its pectin content and characterization.

Results and Discussion

From the results obtained, the pectin yield ranged from 7.30 ± 0.15 % dry wt. to 25.34 ± 0.77 % dry wt. Highest pectin yield was observed in *Citrus medica* fruits i.e. 25.34 ± 0.77 % dry wt. whereas, least amount of pectin yield was observed in *Ficus auriculata* i.e. 5.38 ± 0.13 % dry wt. *Phyllanthus emblica* and

Carissa carandas also contain appreciable amount of pectin i.e. 14.86 ± 0.40 % dry wt. and 11.94 ± 0.60 % dry wt. respectively (Fig.2; Table 2). According to earlier reports, *Citrus* fruits showed 13.91 % of total pectin.[29] Various cultivated fruits like avocado, banana, apple pomace, lemon, mandarin, grapefruit, guava, mango, papaya, pineapple, etc. where pectin value ranged from 0.07- 37.82 % UA.[5] The pectin obtained in recent work showed promising yield than the commercialized fruits.[3,5, 30-35] *Phyllanthus emblica* fruit pectin revealed highest D.E. i.e. 87.63 ± 1.33 % and least D.E. in *Sonneratia caseolaris* i.e. 67.32 ± 0.66 % (Table 2). All the recent studied fruits showed D.E. more than 50.0 % that determine a good gellation capacity.[31] Lemon peels at premature stage had 79.51 % D.E.[32] which was at par with the current result obtained in *Citrus medica* (78.84 ± 2.64 %). According to earlier reports, various other commercialised fruits had been reported for D.E. where, dragon fruit (59.8%), watermelon (64.5 %), mango (80.5%), lemon (85.5 %), grapefruit (90.4 %), banana (75.03 %) and papaya (53.4 %) had been observed.[5,24, 33,34] The gels can be formed by pectin having low methoxyl value using less sugar, absence of sugar in cations (divalent) or no added sugar. The gels obtained from low methoxyl pectin have its individualistic sugar values. A quick set of gel formation is due to high degree of esterification.

Table 2: Pectin yield and its physicochemical properties in 6 wild edible fruits of Odisha

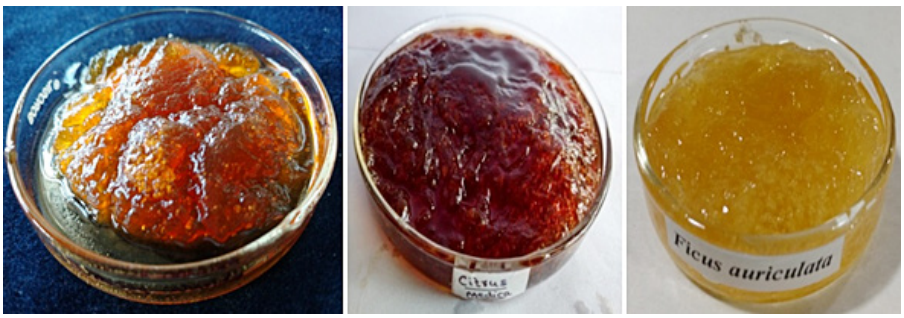
Name of the fruit species	Pectin yield (% dry wt.)	Degree of esterification (%)	Equivalent weight	Methoxyl content (%)	Moisture content (%)	Anhydrouro--nic acid content (%)	P-Value
Carissa carandas	11.94 ± 0.60	78.84 ± 2.64	909.10 ± 31.09	9.92 ± 0.17	4.22 ± 0.25	73.92 ± 4.14	<0.05
Citrus medica	25.34 ± 0.77	85.00 ± 2.48	533.33 ± 16.66	12.74 ± 0.78	10.35 ± 0.35	85.04 ± 2.93	<0.05
Ficus auriculata	5.38 ± 0.13	77.69 ± 0.94	916.66 ± 72.16	11.80 ± 0.25	8.34 ± 0.34	85.20 ± 2.53	<0.05
Limonia acidissima	5.45 ± 0.73	75.00 ± 2.77	808.33 ± 62.91	11.65 ± 1.34	6.25± 0.19	87.86 ± 7.68	<0.05
Phyllanthus emblica	14.86 ± 0.40	87.63 ± 1.33	1516.66 ± 187.63	14.62 ± 0.27	3.84 ± 0.14	94.23 ± 2.67	<0.05
Sonneratia caseolaris	7.30 ± 0.15	67.32 ± 0.66	600.49 ± 21.22	10.64 ± 0.17	7.69 ± 0.29	89.76 ± 1.76	<0.05

*Values expressed as Mean ± standard error (n=3)

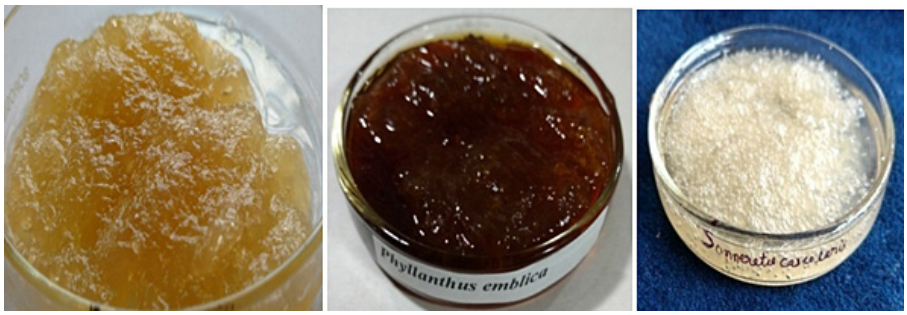
Based on the results acquired in this work, highest equivalent weight was found in *Phyllanthus emblica* (1516.66±187.63) whereas least amount of equivalent weight was observed in *Citrus medica* i.e. 533.33±16.66 (Table 2). The gelling forming effect is based on its equivalent weight where, high equivalent weight denotes high gellation effect.[3] Very few cultivated fruits like orange, sweet lime, papaya, dragon and banana had 625.0, 555.5, 357.2, 713.99 and 1503.16 respectively.[3,33,35] The major property of pectin is its methoxyl value which helps in the production of commercialised fruit jellies. Highest methoxyl content was observed in *Phyllanthus emblica* (14.62±0.27 %) followed by *Citrus medica* (12.74±0.78 %). *Carissa carandas* possessed least amount of methoxyl value (9.92±0.17 %). The results of methoxyl content obtained in *Ficus auriculata* (11.80±0.25 %) and *Limonia acidissima* (11.65±1.34 %)were at par (Table 2). The methoxyl value in several other cultivated fruits like orange (20.15),sweet lime (15.5), papaya (16.85 %) and banana (6.40 %)peels had also been reported earlier by various authors.[3,33] *Citrus medica* wild fruits revealed highest moisture (10.35 ± 0.35 %)and least moisture value exhibited in *Phyllanthus emblica* (3.84 ± 0.14 %) followed by *Carissa carandas* i.e. 4.22 ±

0.25 % (Table 2). The moisture content of pectin present in various marketable fruits like i.e. orange (10.2 %), sweet lime (9.4 %), papaya (8.92 %), banana (14.13 %) and dragon (11.19 %) had been determined.[3,33,35] The resulted equivalent weights was used in the calculation of anhydrouronic acid (% AUA). A low moisture value denotes longer safe storage and helps in microbial growth inhibition which mainly hinders the quality of pectin.[36] AUA plays a crucial role in jellying properties as it determines the purity of pectin. *Phyllanthus emblica* (94.23 ± 2.67 %) exhibited highest percentage of AUA followed by *Sonneratia caseolaris* (89.76 ± 1.76 %)(Table 2). Fruits like orange and mango showed 93.28 % and 89.10 % AUA respectively which was similar to the results obtained in the current study.[37, 38] AUA content of *Carissa carandas* (73.92 ± 4.14 %) was found to be lower than all the present studied fruits but more than the common popular fruits.[33,35] The resulted pectin equivalent to 14 % methoxyl content signifies the degree of esterification ranging from 50 to 80% which is considered as high grade methoxyl pectins whereas, low grade methoxyl pectins having low degree of esterification (< 50 %) and methoxyl content (7%).[39] The polyvalent cations sensitivity, setting time of pectin and helpful in films, fibres and low solid gels is mainly controlled by methoxyl content or degree of esterification. It is mainly regulated by free carboxyl groups and pectin saponification. The AUA content of extracted pectin should be more than 65.0 %.[34]

For statistical analysis, the entire data was analysed in triplicates using ANOVA (one-way) which is followed by a multiple comparison test (Tukey's test). All results showed a significant difference among the mean values of fruit samples. On basis of present work, the confidence limits showed 95 % and the p- value was less than 0.05.



a. *Carissa carandas* **b. *Citrus medica*** **c. *Ficusauriculata***



d. *Limonia acidissima* **e. *Phyllanthus emblica*** **f. *Sonneratia caseolaris***

Fig. 2: (a-f) Pectin production from six wild edible fruits of Odisha

Conclusions

Based on screening and characterization of pectin content, it can be revealed that *Citrus medica*, *Phyllanthus emblica*, *Carissa carandas*, *Sonneratia caseolaris* and *Ficu sauriculata* fruits possess the potential for utilization as raw materials for possible

production of marketable pectins as it is intrinsic, biodegradable, cost effective and absence of toxicity. The results indicated that the pectin yield and its characterization will be useful in preparation of good grade jellies obtained inrespective fruits which can be matter of importance in future food crisis. It can also be benefited to the mankind in diversified ways like food products, industrial and pharmaceutical outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

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