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HRLC-MS analysis of Apong starter culture (E'pob): The starter cake used for the preparation of traditional rice beer of the Mising tribe of Assam

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Abstract

Apong is something other than a drink; the Mising tribe's social, and strict lives have spun on this product. It is made by maturing cooked rice with homemade "E'pob" starter cakes made with a mix of plant parts and rice flour. This alcoholic beverage is possessing many medicinal and therapeutic properties contributed by various indigenous herbs used in starter culture cake preparation. The present investigation was undertaken to analyse bioactive constituents present in "E'pob" by using High Resolution- Liquid Chromatography-Mass Spectrometry (HR-LCMS).

Keywords: Apong, E'pob, Mising, Rice Beer, HRLC-MS

Introduction

Apong, an alcoholic beverage made from fermented rice and local "E'pob" starter cakes. This is an individual part of the Mising people's social, cultural, and religious lives. The peoples of the Mising tribe consumed such preparations after workload daily, they were also used in religious and cultural events. Apong is considered a source of pride for host families and part of their artistic legacy. Alcohol consumption and abstinence are closely tied to core cultural themes, and the forms and meanings associated with its consumption are also significant^[1]. The Mising Tribe uses 39 plant species in Apong preparation, out of these 7-plant species effective for gastrointestinal issues, 6 for skin and respiratory ailments, and 4 for diabetes. Additionally, 2 species are noted for their effects on rheumatism, jaundice, and anti-malarial properties. Starter culture quality depends on plant parts and sanitation. The nutrients in the finished product provide energy, soothing effects, and medical benefits like insomnia, headache, inflammation, diarrhea, urinary problems, worm expulsion, and cholera treatment^[2]. Apong is not just an alcoholic beverage; its biochemical makeup suggests that it is also an alcoholic meal. The palatable final product has various medicinal characteristics and a calming effect, but its nutrients can also give you energy^[3]. The current investigation includes the first report on analysis of Apong Starter Culture by using HRLC-MS.

Materials and Methods

The E'pob was collected from the Lakhimpur District of Assam on 15/08/2023. After proper drying, these cakes were ground with the help of a grinder into a fine powder. The powdered material was kept in airtight condition for further experiments. The extract was prepared from 25 grams of powder by using Soxhlet's extraction method in three different solvents like Ethanol, Petroleum Ether and chloroform. The extract of E'pob was sent to Sophisticated Analytical Instrumentation Facilities (SAIF), IIT Pawai, Mumbai for phytochemical analysis by using HRLC-MS techniques. The qualitative analysis was carried by instrument QTOF, Version: Q-TOF B.05.01 (B5125.3). Many compounds with molecular weight were analysed by this method which belongs to various classes like higher terpenoids, phenolic compounds, alkaloids, flavonoids, lipids, sugars, amino acids etc.

Results and Discussions

The HRLC-MS analysis of Apong starter culture showed presence of 34 compounds as shown in table 1 and 2. The Trolamine is a plant metabolite and act as an antioxidant against the auto-

oxidation [4]; Isopiperolein B [5], Isolimononic acid [6], 2-Hexylbenzothiazole [7] act as a potential biomarker for the consumption of these food; 2-(Methylthiomethyl)-3-phenyl-2-propenal act as a flavouring Agent [8]; Lyso PE induces fatty liver formation [9]; Progeldanamycin is a synthetic glucocorticoid used for its anti-inflammatory or immunosuppressive properties to treat inflammation due to corticosteroid-responsive dermatoses [10]; Phytosphingosine has a number of skin benefits, including barrier function, anti-inflammatory properties, acne management, anti-aging benefits, hydration, and a soothing effect [11]; Sphinganine is an protein kinase C inhibitor (enzyme inhibitor) [12]; Mitoxantrone which has a role as an antineoplastic agent and an analgesic. Mitoxantrone is a Topoisomerase Inhibitor [13] [14, 15]; Phaeophorbide b is a plant metabolite that shows antiviral activities [16] and Cerberin is also a plant metabolite used as medicine in very small amounts to treat cardiac disorders [17].

The compound Carbenicillin is a semisynthetic penicillin. Though carbenicillin provides substantial *in vitro* activity against a variety of both gram-positive and gram-negative microorganisms, the most important aspect of its profile is in its antipseudomonal and antiproteal activity [18]; 2-N,6-N-Bis (2, 3-dihydroxybenzoyl)-L-lysine [19]; Melleolide D [20] are

plant metabolites shows antibacterial activity; Irinotecan is an antineoplastic enzyme inhibitor primarily used in the treatment of colorectal cancer [21]; beta-L-Aspartylhydroxamate act as an antineoplastic agent which inhibits or prevent the proliferation of neoplasms and also an antiviral agent used in the prophylaxis or therapy of virus disease [22, 23]; Protoverine is a compound which structure is of particular interest in view of the potent hypo- tensive action of its ester derivatives and of the use of this antihypertensive action in clinical conditions associated with high blood pressure [24].

The Sulfinpyrazone is a phenylbutazone derivative with uricosuric and antithrombotic properties. Sulfinpyrazone competitively inhibits reabsorption of urate at the proximal renal tubule in the kidney [25]; Sakacin A is a plant metabolite shows antimicrobial activity. Due to antimicrobial effectiveness, sakacin A possesses a significant potential as biopreservative [26] and Mycalamide A is also a plant metabolite shows *in vitro* antiviral activity against A59 coronavirus [27] and Germine may be used medically as an antihypertensive agent. Germine has being shown to have antitumor activity against Hh-dependent lung cancer cell lines, SW1990 and NCI-H249 [28].

Table 1: Positive Compatibility Mode of Apog Starter Culture

Sr. No.	Compound Name	Formula	Group	Mass	RT	DB Diff.(ppm)
1.	Miraxanthin-I	C ₁₄ H ₁₈ N ₂ O ₇ S	Amino acid	358.087	1.428	-9.86
2.	Trolamine	C ₆ H ₁₅ NO ₃	Tertiary Amine	149.1056	4.679	-2.94
3.	3-[(3- Methylbutyl) nitrosoamino]-2-butanone	C ₉ H ₁₈ N ₂ O ₂	Nitrosamine	186.1351	5.637	9.2
4.	Isopiperolein B	C ₂₁ H ₂₉ NO ₃	Benzodioxoles	343.2177	6.667	-8.64
5.	Progeldanamycin	C ₂₇ H ₄₁ NO ₆	Lactam and an azamacrocycle	475.2953	7.637	-4.04
6.	14 alpha-Hydroxypaxilline	C ₂₇ H ₃₃ NO ₅	Naphthopyrans	451.2379	11.93	-4.56
7.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	Amino cyclitol and a glycoside	567.285	12.831	2.47
8.	D-Linalool 3-(6"-malonylglucoside)	C ₁₉ H ₃₀ O ₉	O-acyl carbohydrate	402.1904	14.25	-3.46
9.	Phytosphingosin	C ₁₈ H ₃₉ NO ₃	Amino alcohol	317.2904	14.796	8.29
10.	Sphinganine	C ₁₈ H ₃₉ NO ₂	Amino alcohol	301.2951	15.706	10
11.	Mitoxantrone	C ₂₂ H ₂₈ N ₄ O ₆	Dihydroxy anthraquinone	444.2012	16.187	-0.72
12.	Cerbertin	C ₃₂ H ₄₄ O ₁₁	Glycoside	604.2868	16.701	2.52
13.	Isolimononic acid	C ₂₆ H ₃₄ O ₁₀	Naphthofuran	506.2166	18.276	-2.87
14.	LysoPE(0:0/16:0)	C ₂₁ H ₄₄ NO ₇ P	Lysophospholipid	453.2815	18.733	8.85
15.	Oxidized dinoflagellate luciferin	C ₃₃ H ₃₈ N ₄ O ₇	Bilenes	602.2719	19.911	3.49
16.	D-Urobilin	C ₃₃ H ₄₀ N ₄ O ₆	Biladienes	588.2926	20.321	3.75
17.	PE (14:1(9Z)/14:0)	C ₃₃ H ₆₄ NO ₈ P	Phosphatidylethanolamine	633.44	20.753	-4.75
18.	Phaeophorbide b	C ₃₅ H ₃₄ N ₄ O ₆	Tetrapyrrole	606.2429	22.704	8.15
19.	Irinotecan	C ₃₃ H ₃₈ N ₄ O ₆	Pyranoindolizinoquinoline	586.2764	23.931	4.63
20.	Harderoporphyrin	C ₃₅ H ₃₆ N ₄ O ₆	Porphyrins	608.2581	24.445	8.86

Table 2: Negative Compatibility Mode of Apog Starter Culture

Sr. No.	Compound Name	Formula	Group	Mass	RT	DB Diff. (ppm)
1.	Carbenicillin	C ₁₇ H ₁₈ N ₂ O ₆ S	Semisynthetic penicillin	378.0881	1.451	1.13
2.	2-(Methylthiomethyl)-3-phenyl-2-propenal	C ₁₁ H ₁₂ OS	Cinnamaldehydes	192.0602	1.498	3.35
3.	2-Hexylbenzothiazole	C ₁₃ H ₁₇ NS	Benzothiazoles	219.1068	6.379	6.1
4.	beta-L-Aspartylhydroxamate	C ₄ H ₈ N ₂ O ₄	Amino acid	148.0493	9.237	-6.13
5.	Protoverine	C ₂₇ H ₄₃ NO ₉	Alkaloids	525.2906	15.05	6.06
6.	LysoPE (22:5 (4Z,7Z,10Z,13Z,16Z) /0:0)	C ₂₇ H ₄₆ NO ₇ P	Lysophosphatidylethanolamines	527.3065	16.045	-10
7.	Sulfinpyrazone	C ₂₃ H ₂₀ N ₂ O ₃ S	Pyrazolidines	404.1214	16.538	-4.69
8.	Mitoxantrone	C ₂₂ H ₂₈ N ₄ O ₆	Dihydroxy anthraquinone	444.1999	16.628	2.2
9.	2-N,6-N-Bis (2,3- dihydroxybenzoyl)-L-lysine	C ₂₀ H ₂₂ N ₂ O ₈	N-acylglycine	418.1367	17.423	2.13
10.	Sakacin A	C ₁₂ H ₂₄ N ₄ O ₄	Aspartic acid derivative	288.1785	17.478	4.47
11.	Mycalamide A	C ₂₄ H ₄₁ NO ₁₀		503.2704	17.921	5.22
12.	Istamycin C1	C ₁₉ H ₃₇ N ₅ O ₆	Formamides	431.2743	18.157	0.1
13.	Melleolide D	C ₂₄ H ₃₁ Cl O ₈	Sesquiterpenoids	482.1699	18.652	1.7
14.	Germine	C ₂₇ H ₄₃ NO ₈	Alkaloids	509.2954	19.691	6.81

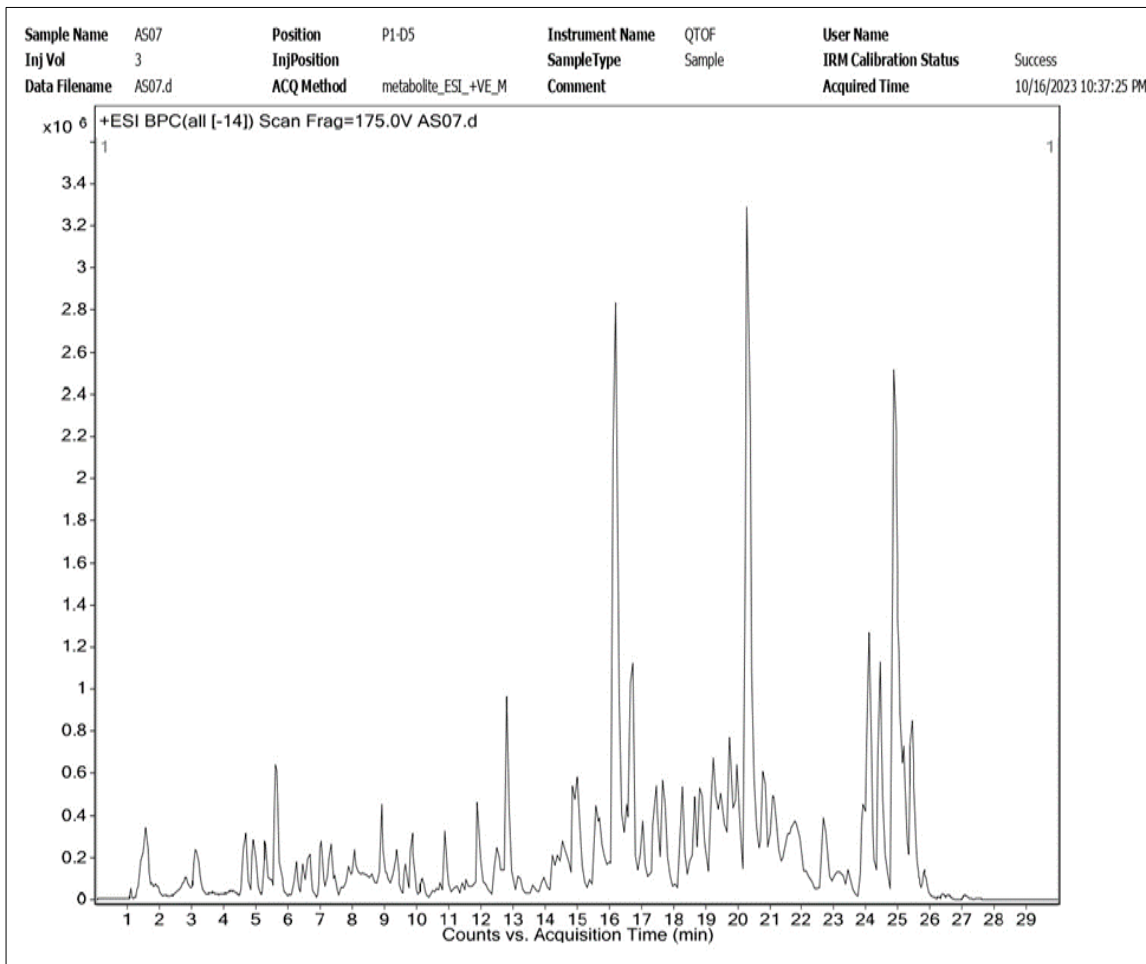


Fig 1: Chromatogram for Positive Mode of Apong Starter Culture

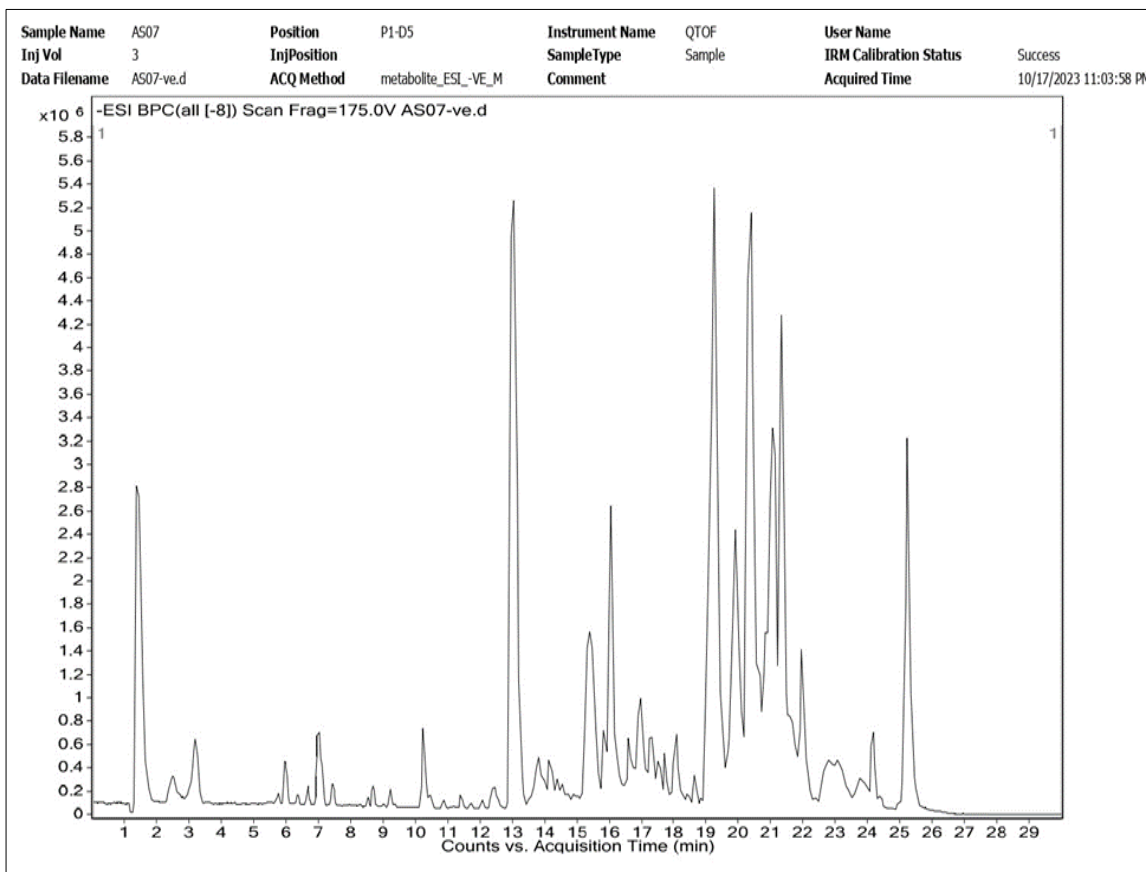


Fig 2: Chromatogram for Negative Mode of Apong Starter Culture

Conclusion

The ethanolic extract of Apong Starter Cake (E'pob) revealed the presence of important phytochemical compounds like Trolamine, Isopiperolein B, Progeldanamycin, Phytosphingosin, Sphinganine, Mitoxantrone, Cerberin, Isolimononic acid, LysoPE, Phaeophorbide b, Irinotecan, Carbenicillin, 2-(Methylthiomethyl)-3-phenyl-2-propenal, 2-Hexylbenzothiazole, beta-L-Aspartylhydroxamate, Protoverine, Sulfinpyrazone, 2-N,6-N-Bis(2,3-dihydroxybenzoyl)-L-lysine, Sakacin A, Mycalamide A, Melleolide D Germine, using high-resolution liquid chromatography-mass spectrometry (HRLC-MS) analysis. The E'pob shows anti-inflammatory, antiviral, antibacterial, antimicrobial, antitumor activities and also use for to treat cardiac disorders.

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