

GC-MS Analysis of Bio-active Compounds in Ethanol Extract of *Putranjiva roxburghii* Wall. Fruit Peel

Md. Mahmudul Hasan*, Md. Rezuan Al Mahmud, Md. Gaziul Islam

ABSTRACT

Introduction: *Putranjiva roxburghii* Wall. has long been used in folkloric medicine for treating arthralgia, fever, hemorrhoids, muscle pain and rheumatism. But no reports were found regarding phytochemical constituents in *P. roxburghii* fruit peel. Therefore, this study was designed to analyze extract of *P. roxburghii* fruit peel (PRFP). **Methods:** Gas chromatography-mass spectrometry (GC-MS) analysis of the ethanol extract of PRFP was carried out by using a GC-MS equipment. **Results:** The GC-MS analysis has revealed the existence of different phytochemical compounds in the ethanolic extract of PRFP. The major compounds in PRFP extract are Cyclohexanol, 5-methyl-2-(1-methylethenyl)- (4.56%), 6-Octen-1-ol, 3,7-dimethyl- (41.07%), Geraniol (2.45%), (1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol (14.09%), 2,6-Octadiene, 2,6-dimethyl- (7.04%), p-Menthane-3,8-diol, cis-1,3,trans-1,4- (3.39%), 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate (6.69%) and 13-Docosenamido, (Z)- (2.83%). A total of 25 compounds identified representing 99.98% of total ethanolic extract. **Conclusion:** Overall finding suggests that PRFP contain various phytochemicals and is recommended as an important source of pharmaceutical ingredients.

Key words: *Putranjiva roxburghii*, Fruit peel, Ethanol extract, GC-MS, Pharmaceutical ingredients.

Md. Mahmudul Hasan*,
Md. Rezuan Al Mahmud,
Md. Gaziul Islam

Molecular Biology and Protein Science
Laboratory, Department of Genetic
Engineering and Biotechnology, Faculty
of Life and Earth Sciences, University of
Rajshahi, Rajshahi-6205, BANGLADESH.

Correspondence

Mr. Md. Mahmudul Hasan

Molecular Biology and Protein Science
Laboratory, Department of Genetic
Engineering and Biotechnology, Faculty
of Life and Earth Sciences, University of
Rajshahi, Rajshahi-6205, BANGLADESH.

Phone no : +8801720214658

E-mail: mahmudul_geb@yahoo.com

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INTRODUCTION

Putranjiva roxburghii Wall. belongs to the *Euphorbiaceae* family. It is widely grown in Bangladesh, India, Indochina, Myanmar, Nepal, Sri Lanka and Thailand.¹ It is locally known as “Putranjiv” in Bangladesh. This is an ornamental plant of tropical India and known as child life tree.² The seed of this plant is a good source of well stable and potent trypsin inhibitor.² The seed is also a potent source of thermostable glycosyl hydrolase family 1 enzyme with β -D-glucosidase and β -D-galactosidase activities.³ Spreading the leaves of this plant over the floor of maternity room has been reported for an easy delivery.⁴ In Thailand, *P. roxburghii* fruits and leaves have long been traditionally used for the treatment of arthralgia, fever, muscle pain and rheumatism and the entire plant has also been used for the treatment of hemorrhoids and fever.^{1,5} *P. roxburghii* leaf has analgesic, anti-inflammatory and antipyretic activities.⁶ Though *P. roxburghii* has long been used in folkloric treatment, no studies have been conducted regarding the presence of phytochemicals in *P. roxburghii* fruit peel. Thereafter, this current study was aimed to investigate the bioactive phytochemicals in the fruit peel extract of *P. roxburghii*.

MATERIALS AND METHODS

Chemicals and reagents

All of the chemicals and reagents used in this study were of analytical grade.

Collection of plant material

The fruit of *Putranjiva roxburghii* plant was collected from the University of Rajshahi (Rajshahi, Bangladesh) campus in October 2017. Then the fruit was identified and authenticated by a taxonomist Mr. Md. Arshed Alam (Department of Botany, University of Rajshahi, Rajshahi, Bangladesh). A herbarium of the *P. roxburghii* containing fruit was prepared and preserved in the Herbarium of the Department of Botany, University of Rajshahi, Rajshahi, Bangladesh under an accession number 00312 for further referencing.

Preparation of extract

The peel was separated from the fruits and was dried by using a drying cabinet (LJ-120A(S), Guangdong LIK Industry Co., Ltd., China) at 37°C temperature. The dried peel was grinded to make fine powder. Then extraction was performed by dissolving the powder (5 g) in 50 ml of 95% ethanol. The content was sonicated by using sonicator (Soniprep 150, China) at 20 kHz for 10 min. Extract was filtered using Glass Fiber Filter paper (Macherey NAGEL, GmbH, German) with DURAN® Filtering Apparatus (German) at room temperature. The resulting solution was concentrated with VirTis BenchTop Pro Freeze Dryer (German). Finally, the extract was stored in a refrigerator at 4°C for further use.

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GC-MS analysis

The phytochemical investigation of ethanolic extract was carried out on a GC-MS equipment (GCMS-QP2020; SHIMADZU) comprising an AOC-20s auto-sampler, an AOC-20i auto-injector and a Gas Chromatograph (GC-2010 Plus) interfaced to a Mass Spectrometer. Experimental conditions of GC-MS system were as follows: SH-Rxi-5Sil-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, film thickness: 0.25 μ m. Flow rate of mobile phase (carrier gas: He) was set at 1.7 ml/min. In the gas chromatography part, temperature profile (initial temperature) was 80°C (hold time 2.0 min) rose to 150°C at 5°C/min (hold time 5.0 min) and finally the temperature was increased to 280°C at 5°C (hold time 8.0 min). The injection volume was 5 μ l. Sample dissolved in methanol was run at a range of 45-350 m/z and the results were compared by using National Institute of Standards and Technology such as NIST08, NIST08s and NIST14 Library Search Programmes.

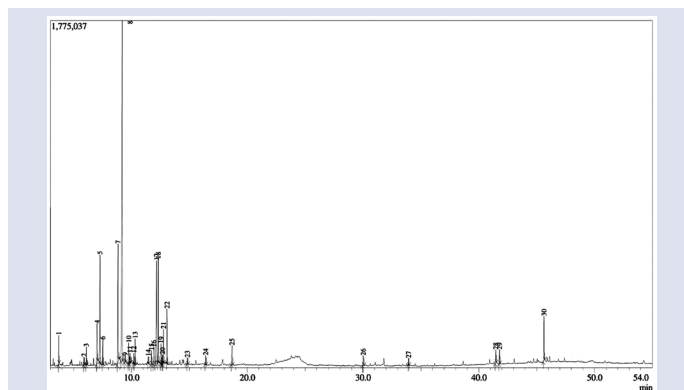


Figure 1: GC-MS chromatogram of PRFP ethanol extract.

Table 1: List of compounds in PRFP ethanol extract identified by using GC-MS.

Com. no.	Peak no.	Name of compounds	Ret. time (min)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
1	1	3,4-Hexanediol, 2,5-dimethyl-	3.714	C ₈ H ₁₈ O ₂	146.227	1.53
2	2	Linalool	5.890	C ₁₀ H ₁₈ O	154.253	0.45
	3	Linalool	6.094	C ₁₀ H ₁₈ O	154.253	0.89
3	4	Citronellal	7.016	C ₁₀ H ₁₈ O	154.253	1.86
4	5	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	7.275	C ₁₀ H ₁₈ O	154.253	3.97
	6	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	7.514	C ₁₀ H ₁₈ O	154.253	0.59
5	7	6-Octen-1-ol, 3,7-dimethyl-	8.829	C ₁₀ H ₂₀ O	156.265	8.53
	8	6-Octen-1-ol, 3,7-dimethyl-	9.192	C ₁₀ H ₂₀ O	156.265	32.54
6	9	2,6-Octadienal, 3,7-dimethyl-, (Z)-	9.445	C ₁₀ H ₁₆ O	152.237	0.43
7	10	Geraniol	9.756	C ₁₀ H ₁₈ O	154.253	2.45
8	11	4-Isopropyl-1,3-cyclohexanedione	9.851	C ₉ H ₁₄ O ₂	154.209	0.50
9	12	2,6-Octadienal, 3,7-dimethyl-, (E)-	10.192	C ₁₀ H ₁₆ O	152.237	1.19
10	13	6-Octen-1-ol, 3,7-dimethyl-, formate	10.319	C ₁₁ H ₂₀ O ₂	184.279	1.65
11	14	(R)-(+)-Citronellic acid	11.453	C ₁₀ H ₁₈ O ₂	170.252	0.70
12	15	(1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol	11.723	C ₁₀ H ₂₀ O ₂	172.265	1.17
13	16	2,6-Octadiene, 2,6-dimethyl-	11.933	C ₁₀ H ₁₈	138.254	1.46
12	17	(1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol	12.169	C ₁₀ H ₂₀ O ₂	172.265	12.92
13	18	2,6-Octadiene, 2,6-dimethyl-	12.317	C ₁₀ H ₁₈	138.254	5.58
14	19	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	12.549	C ₁₂ H ₂₀ O ₂	196.290	1.90
15	20	Geranyl acetate	12.670	C ₁₂ H ₂₀ O ₂	196.290	1.33
16	21	p-Menthane-3,8-diol, cis-1,3,trans-1,4-	12.759	C ₁₀ H ₂₀ O ₂	172.268	3.39
17	22	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	13.062	C ₁₂ H ₂₀ O ₂	196.290	6.69
18	23	3-Hydroxy-4-methoxybenzyl alcohol	14.820	C ₈ H ₁₀ O ₃	154.165	0.38
19	24	β -Bisabolene	16.400	C ₁₅ H ₂₄	204.357	0.49
20	25	Caryophyllene oxide	18.685	C ₁₅ H ₂₄ O	220.356	0.90
21	26	Hexadecanoic acid, methyl ester	30.005	C ₁₇ H ₃₄ O ₂	270.457	0.79
22	27	11-Octadecenoic acid, methyl ester	33.929	C ₁₉ H ₃₆ O ₂	296.495	0.33
23	28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	41.466	C ₁₉ H ₃₈ O ₄	330.509	0.66
24	29	Bis(2-ethylhexyl) phthalate	41.790	C ₂₄ H ₃₈ O ₄	390.564	1.88
25	30	13-Docosamide, (Z)-	45.631	C ₂₂ H ₄₃ NO	337.592	2.83

Table 2: Bio-activity of compounds identified in PRFP ethanol extract by GC-MS.

Com. no.	Name of compounds	Bio-activity
1	3,4-Hexanediol, 2,5-dimethyl-	Not reported
2	Linalool	Anticancer, antimicrobial, anti-inflammatory, anti-hyperlipidemic, antinoceptive, analgesic, anxiolytic, antidepressive and neuroprotective ⁷
3	Citronellal	Antioxidant, antimicrobial, anthelmintic, anticonvulsant antitrypanosomal, wound healing and mosquito repellent ⁸
4	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	Antimicrobial, anticancer, anti-tumor, analgesic, anti-inflammatory, sedative, antifungal, hypocholesterolemic, insecticide, insectifuge chemo preventive and pesticidal ⁹
5	6-Octen-1-ol, 3,7-dimethyl-	Antinociceptive, anti-inflammatory, antifungal, repellent and larvicidal ¹⁰
6	2,6-Octadienal, 3,7-dimethyl-, (Z)-	Antimicrobial ¹¹
7	Geraniol	Anticancer, antibacterial, anthelmintic, anti-inflammatory and anticarcinogenic ¹²
8	4-Isopropyl-1,3-cyclohexanedione	Not reported
9	2,6-Octadienal, 3,7-dimethyl-, (E)-	Antimicrobial ¹¹
10	6-Octen-1-ol, 3,7-dimethyl-, formate	Antitumor, antibacterial, antifungal, flavoring and fragrance ¹³⁻¹⁶
11	(R)-(+)-Citronellic acid	Anticancer ¹⁷
12	(1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol	Anticancer, antimicrobial, anti-inflammatory and flavour enhancing ¹⁸
13	2,6-Octadiene, 2,6-dimethyl-	Not reported
14	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	Anticancer and antinociceptive ^{19,20}
15	Geranyl acetate	Anticancer and antinociceptive ^{19,20}
16	p-Menthane-3,8-diol, cis-1,3,trans-1,4-	Mosquito repellent ²¹
17	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	Anticancer ²⁰
18	3-Hydroxy-4-methoxybenzyl alcohol	Not reported
19	β -Bisabolene	Anticancer ²²
20	Caryophyllene oxide	Anticancer, antioxidant, anti-inflammatory immunomodulator, cytoprotective and trypanocidal ²³
21	Hexadecanoic acid, methyl ester	Antioxidant, antibacterial and antifungal ^{24,25}
22	11-Octadecenoic acid, methyl ester	Not reported
23	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Antioxidant, anti-inflammatory, anthelmintic ²⁵
24	Bis(2-ethylhexyl) phthalate	Cytotoxic ²⁶
25	13-Docosamide, (Z)-	Antiviral ²⁶

RESULTS AND DISCUSSION

The GC-MS Chromatogram revealed a total of 30 peaks with different retention times (Figure 1). Though the peak number is 30, the identified compounds are 25 due to the reiteration of 5 compounds (Table 1). The large compound fragments compared to small compounds gave taller appearance of peaks at different m/z ratios.

However, the identified compounds in the extract of PRFP are 3,4-Hexanediol,2,5-dimethyl-, Linalool, Citronellal, Cyclohexanol,5-methyl-2-(1-methylethenyl)-, 6-Octen-1-ol,3,7-dimethyl-, 2,6-Octadienal,3,7-dimethyl-,(Z)-, Geraniol, 4-Isopropyl-1,3-cyclohexanedione, 2,6-Octadienal, 3,7-dimethyl-,(E)-, 6-Octen-1-ol,3,7-dimethyl-, formate, (R)-(+)-Citronellic acid, (1R,2S,5R)-2-(2-Hydroxy-2-propanyl)-5-methylcyclohexanol, 2,6-Octadiene,2,6-dimethyl-, 2,6-Octadien-1-ol,3,7-dimethyl-,acetate,(Z)-, Geranyl acetate, p-Menthane-3,8-diol,cis-1,3,trans-1,4-, 2,6-Octadien-1-ol,3,7-dimethyl-,acetate, 3-Hydroxy-4-methoxybenzyl alcohol, β -Bisabolene, Caryophyllene oxide, Hexadecanoic acid, methyl ester, 11-Octadecenoic acid, methyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Bis(2-ethylhexyl) phthalate and 13-Docosamide, (Z)- (Table 1). The composition determined for the PRFP

extract corresponds to 99.98% of the whole GC-MS chromatogram. The identified compounds were previously reported with numerous important bio-activities (Table 2).

CONCLUSION

The identified compounds after GC-MS analysis of *P. roxburghii* fruit peel extract justifies the use of fruit peel by traditional practitioner. However, isolation of each single compounds and subjecting it to the biological activity will definitely give fruitful outcomes as the identified compounds were previously reported with important bio-activity. Therefore, this plant is recommended as a source of phytopharmaceutical value.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

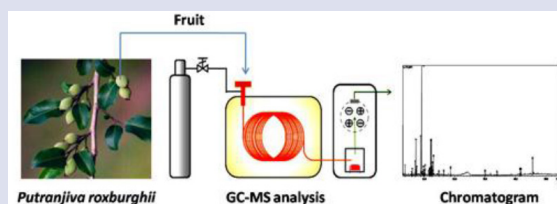
ABBREVIATIONS

GC-MS: Gas chromatography-Mass spectrometry; **PRFP:** *P. roxburghii* Fruit Peel.

REFERENCES

- Phupathanaphong L. Flora of Thailand *Euphorbiaceae*. Natl Herb Nederland. 2006;336:1877-87.
- Chaudhary NS, Shee C, Islam A, Ahmad F, Yernool D, Kumar P, *et al.* Purification and characterization of a trypsin inhibitor from *Putranjiva roxburghii* seeds. *Phytochemistry*. 2008;69(11):2120-6.
- Patel GK, Kar B, Sharma AK. Characterization of a thermostable family 1 Glycosyl Hydrolase enzyme from *Putranjiva roxburghii* seeds. *Applied Biochemistry and Biotechnology*. 2012;166(3):523-35.
- Singh H, Bisht GS. Some novel folk treatments among the Tribes of Uttar Pradesh. *Ancient science of Life*. 1999;18(3-4):250-53.
- Boonyaprapat N, Chokechaicharoenporn A. Samunprai Maipuenban. Prachachon, Bangkok. 1999;3:262-73.
- Reanmongkol W, Noppapan T, Subhadhirasakul S. Antinociceptive, antipyretic, and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. *Journal of Natural Medicines*. 2009;63(3):290-9.
- Pereira I, Severino P, Santos AC, Silva AM, Souto EB. Linalool Bioactive Properties and Potential Applicability in Drug Delivery Systems. *Colloids and Surfaces B: Biointerfaces*. 2018.
- Sharma R, Rao R, Kumar S, Mahant S, Khatkar S. Therapeutic potential of Citronella Essential Oil: a review. *Current Drug Discovery Technologies*. 2018.
- Rency RC, Vasantha K, Maruthasalam A. Identification of bioactive compounds from ethanolic leaf extracts of *Premnas ratifolia* L. using GC-MS. *Bioscience Discovery*. 2015;6(2):96-101.
- Pereira FD, Mendes JM, Lima IO, Mota KS, Oliveira WA, Lima ED. Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against *Trichophyton rubrum* involves inhibition of ergosterol biosynthesis. *Pharmaceutical Biology*. 2015;53(2):228-34.
- Dubey D, Patnaik R, Ghosh G, Padhy RN. *In vitro* Antibacterial Activity, Gas Chromatography–Mass Spectrometry Analysis of *Woodfordia fruticosa* Kurz. Leaf Extract and Host Toxicity Testing With *In vitro* Cultured Lymphocytes From Human Umbilical Cord Blood. *Osong Public Health and Research Perspectives*. 2014;5(5):298-312.
- Singh S, Fatima Z, Ahmad K, Hameed S. Fungicidal action of geraniol against *Candida albicans* is potentiated by abrogated CaCdr1p drug efflux and fluconazole synergism. *PLoS One*. 2018;13(8):e0203079.
- Fang HJ, Su XL, Liu HY, Chen YH, Ni JH. Studies on the chemical components and anti-tumour action of the volatile oils from *Pelargonium graveoleus*. Yao Xuexuebao = *Acta Pharmaceutica Sinica*. 1989;24(5):366-71.
- Rath CC, Dash SK, Rao BR. Antifungal activity of rose-scented geranium (*Pelargonium species*) essential oil and its six constituents. *Journal of Essential Oil Bearing Plants*. 2005;8(2):218-22.
- Choi HS. Characteristic odor components of kumquat (*Fortunella japonica* Swingle) peel oil. *Journal of Agricultural and Food Chemistry*. 2005;53(5):1642-7.
- Bigos M, Wasiela M, Kalembe D, Sienkiewicz M. Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus*. *Molecules*. 2012;17(9):10276-91.
- Rychlicka M, Niezgodna N, Gliszczynska A. Lipase-Catalyzed Acidolysis of Egg-Yolk Phosphatidylcholine with Citronellic Acid. New Insight into Synthesis of Isoprenoid-Phospholipids. *Molecules*. 2018;23(2):314.
- Kamatou GP, Vermaak I, Viljoen AM, Lawrence BM. Menthol: a simple monoterpene with remarkable biological properties. *Phytochemistry*. 2013;96:15-25.
- Quintans-Júnior L, Moreira JC, Pasquali MA, Rabie S, Pires AS, Schröder R, *et al.* Antinociceptive activity and redox profile of the monoterpenes. *ISRN Toxicology*. 2013.
- Qi F, Yan Q, Zheng Z, Liu J, Chen Y, Zhang G. Geraniol and geranyl acetate induce potent anticancer effects in colon cancer Colo-205 cells by inducing apoptosis, DNA damage and cell cycle arrest. *Journal of BUON: Official Journal of the Balkan Union of Oncology*. 2018;23(2):346-52.
- Webb CE, Hess IM. A review of recommendations on the safe and effective use of topical mosquito repellents. *Public Health Res Pract*. 2016;26(5):e2651657.
- Yeo SK, Ali AY, Hayward OA, Turnham D, Jackson T, Bowen ID, *et al.* β -Bisabolene, a Sesquiterpene from the Essential Oil Extract of *Opoponax (Commiphora guidottii)*, Exhibits Cytotoxicity in Breast Cancer Cell Lines. *Phytotherapy Research*. 2016;30(3):418-25.
- Moreno ÉM, Leal SM, Stashenko EE, García LT. Induction of programmed cell death in *Trypanosoma cruzi* by *Lippia alba* essential oils and their major and synergistic terpenes (citral, limonene and caryophyllene oxide). *BMC Complementary and Alternative Medicine*. 2018;18(1):225.
- Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portula castrum* L. *European Review for Medical and Pharmacological Sciences*. 2011;15(7):775-80.
- Chen Y, Zhou D, Qi D, Gao Z, Xie J, Luo Y. Growth Promotion and Disease Suppression Ability of a *Streptomyces* sp. CB-75 from Banana Rhizosphere Soil. *Frontiers in Microbiology*. 2018;8:2704.
- Wang Y, Ren W, Li Y, Xu Y, Teng Y, Christie P, *et al.* Non targeted metabolomic analysis to unravel the impact of di (2-ethylhexyl) phthalate stress on root exudates of alfalfa (*Medicago sativa*). *Science of the Total Environment*. 2019;646:212-9.

GRAPHICAL ABSTRACT



SUMMARY

- The bioactive constituents of ethanol extract of *Putranjiva roxburghii* fruit peel was evaluated using GC-MS. A total of 25 compounds were identified and the most abundant compound is 6-Octen-1-ol, 3,7-dimethyl- (41.07%). However, fruit peel extract from this plant may become as a good source of new drugs for therapeutic studies.

ABOUT AUTHORS



Md. Gaziul Islam: Is an undergraduate research student who has a big desire on Molecular Microbiology.



Md. Mahmudul Hasan: Is a research personal who is searching for sources of natural and safe product that may have potent therapeutic effect to combat against cancer. Other areas of his research interest are Molecular Pathology, Redox Regulation, Drug Design and Nutrigenomics.



Md. Rezuhan Al Mahmud: Currently working as a Scientific Officer in Globe Biotech Limited (Bangladesh) which focuses on the Drug Design and Development. He has great interest on Molecular Biology and Drug Design.

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