International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(4): 3027-3035

Received: 16-05-2019
Accepted: 18-06-2019

A Nathira Fathima
Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

D Uma
Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

R Parimala Devi
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Velmathi
Department of Chemistry, National Institute of Technology, Tiruchirappalli, Tamil Nadu, India

JA Banday
Department of Chemistry, National Institute of Technology, Srinagar, Jammu and Kashmir, India

VP Santhanakrishnan
Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
VP Santhanakrishnan
Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Microbial transformation of heraclenin and osthol isolated from the root extracts of *Prangos pabularia*

A Nathira Fathima, D Uma, R Parimala Devi, S Velmathi, JA Banday and VP Santhanakrishnan

Abstract
Osthol, Heraclenin and Oxypeucedanin were isolated from the hexane extract of *Prangos pabularia* and the structure was confirmed by NMR spectroscopy. The biotransformation of osthol epoxide and Heraclenin was performed using *Saccharomyces cerevisiae*. The structure of these compounds were analysed by NMR spectroscopy and mass spectroscopy. The result reveals that the reduction has taken by in epoxides by ring opening of the epoxide.

Keywords: *Prangos pabularia*, biotransformation, osthol and heraclenin

Introduction
Prangos is the most widely distributed and important genus in the Umbelliferae family consisting of around 36 species worldwide [1].In India, they are commonly known as "Komal" and occurs in the rocky slopes of Jammu and Kashmir [1]. There are various coumarins, flavonoids, alkaloids and terpenoids were isolated from various *Prangos* species [3, 7]. The extracts and the compounds are known to possess many biological activities including antibacterial [4, 6], antispasmodic effects [8, 9]. Anti-inflammatory effects [6]. Antioxidant [10, 11]. Insecticidal activities [12]. And cytotoxicity effects [11, 13]. The chloroform root extract of this plant yielded yuganin A (7-methoxy-8-((1S,2S)-1,2,3-trihydroxy-3-methylbutyl)-2H-chromen-2-one), heraclenol 3′-O-β-D-glucopyranoside, oxypeucedanin hydrate 3′-O-β-D-glucopyranoside, heraclenol, oxypeucedanin hydrate, osthol, oxypeucedanin, heraclenin, isoimperatorin, imperatorin and the disaccharide [14]. The biological activities of these compounds can be enhanced by biotransformation. The microorganisms have the capability to chemical modify a wide range of organic compounds. During the biotransformation process, they synthesize many enzymes which act on organic compounds and modify them. Biotransformation processes are mediated by two groups of microorganisms especially bacteria and fungi [15]. The whole-cell biocatalysts are more convenient and more stable sources of enzymes and are often preferred. These cells have a high capacity to act as a redox biocatalyst [16]. *Saccharomyces cerevisiae* has been recurrently employed in biotransformation reactions, due to its readiness of access and low cost. They are the ideal choice for conversions that lead to the oxidation-reduction and has a long history of safe use in the food industry makes this microorganism user-friendly [17, 18]. Dehydrogenases have been extensively used for the reduction of carbonyl groups of aldehydes or ketones and carbon-carbon double bonds. *Saccharomyces cerevisiae* helps in the asymmetric reduction of carbonyl compounds [19]. In this study, biotransformation of compounds were carried out using *Saccharomyces cerevisiae* as they were able to attack the epoxide ring present in heraclenin and osthol epoxide to get the new derivatives of the parent compound. The structures of these compounds were elucidated using extensive spectral data.

Materials and Methods
Sample collection
The roots of *Prangos pabularia* were collected from Kashmir and the plant was identified by Dr. J.A. Banday, Department of Chemistry, National Institute of Technology, Srinagar, Jammu and Kashmir.

Compound extraction

Procedure for the extraction of Heraclenin, Osthol and Oxypeucedanin

The chemicals used for this research was procured from Sigma- Aldrich. The roots of *Prangos pabularia* were shade dried and powdered using a mixer. Then 50 g of roots were taken in a glass jar and 200 ml of Hexane was added and left overnight (2x). The solvent was then filtered using filter paper and kept for slow evaporation. 1.883 g of crude extract obtained was purified by column chromatography using Silica gel 70-230 mesh as stationary phase and eluted with solvents of increasing polarity. The isolated compounds were confirmed by performing thin layer chromatography on 0.25 mm silica gel F254 plates (Merck) and UV was used to visualize the spots.

A Simple procedure for the extraction of oxypeucedanin

Oxypeucedanin was extracted by keeping the hexane extract @ 4 °C. The sediment formed was air dried to get oxypeucedanin. The isolated compound was confirmed by performing thin layer chromatography and NMR spectroscopy.

Biotransformation of heraclenin

100 mg of Heraclenin added to the medium containing 1333.32mg of *Saccharomyces cerevisiae* and 5 g of sucrose and incubated for 15 days @ 28 °C. The transformed compound was sequentially extracted using DCM solvent by keeping in the shaker for 10 min. This process was followed thrice and the extract was pooled. The trace of water present was removed by adding sodium sulphate crystals. The solvent was removed to afford the crude extract of the transformed compound. The transformed compound was purified by column chromatography and analysed using NMR spectroscopy.

Chemical transformation of osthol

For chemically transforming the osthol, 120 mg of osthol was treated with 200 mg of 3- perchloro benzoic acid and 4 ml of Dichloromethane was added and kept in the shaker for 3 hours. The compound was extracted by after adding 300mg of sodium bicarbonate to neutralize the acid. Then it was washed with water and DCM. The filtrate was extracted with DCM and dried using little quantity of sodium sulphate and evaporated on hot plate to give a yield of 146 mg. The compounds were purified by column chromatography using Silica gel 70-230 mesh as a stationary phase. The isolated compounds were confirmed by performing thin layer chromatography on 0.25 mm silica gel F254 plates (Merck) and UV was used to visualize the spots and further confirmed by NMR spectroscopy.

Biotransformation of osthol epoxide

For chemically transforming the osthol, 30 mg of epoxy osthol added to the medium containing 833.33 mg of *Saccharomyces cerevisiae* and 2 g of sucrose and incubated for 7 days @ 28 °C. The transformed compound was sequentially extracted using DCM solvent by keeping in the shaker for 10 min. This process was followed thrice and the extract was pooled. The traces of water present were removed by adding sodium sulphate crystals. The solvent was removed to afford the crude extract of the transformed compound. The transformed compound was purified by column chromatography and analysed using NMR spectroscopy.

Result and Discussion

Isolation of compounds

The compounds isolated were found to be osthol, heraclenin and oxypeucedanin which were confirmed using NMR spectroscopy. Similar results regarding the presence of these compounds in the Prangos species were confirmed by the previous research [14].

NMR of heraclenin: White crystals; Yield: 126 mg.

^{1}H NMR: 7.71(d,1H,J=9.5Hz), 7.69(d,1H,J=2.0Hz), 7.40 (s,1H), 7.26(s,1H), 6.82(d,1H,J=2.0Hz), 6.36(d,1H,J=9.5Hz), 4.58(m,2H), 3.31(t,1H,J=5.5Hz), 1.34(s,3H), 1.28(s,3H).

^{13}C NMR: 160.47, 148.44, 146.94, 144.45, 143.74, 131.57, 126.12, 116.63, 114.92, 114.01, 106.94, 106.10, 72.59, 61.45, 58.30, 24.68, 18.99.

The NMR spectral data of the heraclenin (Fig: 1) were compared with those of previously reported data and it was proved to be identical which confirms the isolated compounds [20].

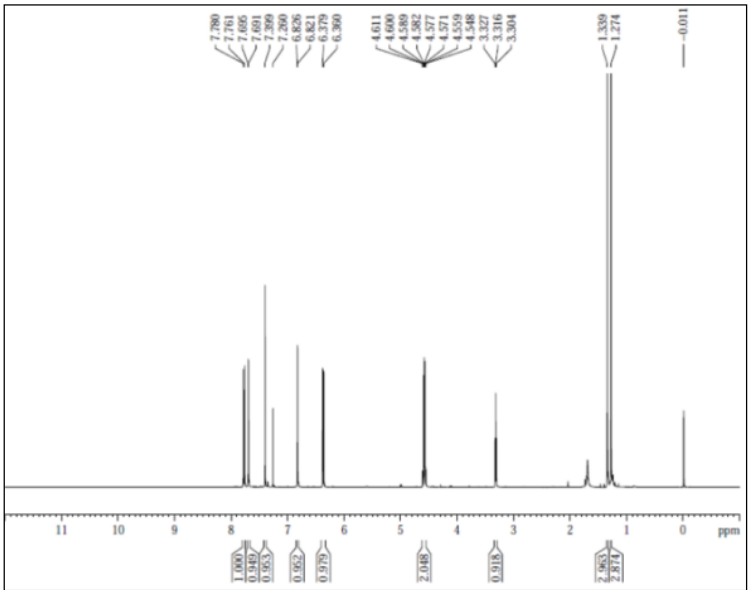

Fig 1(a): 1 H NMR of Heraclenin

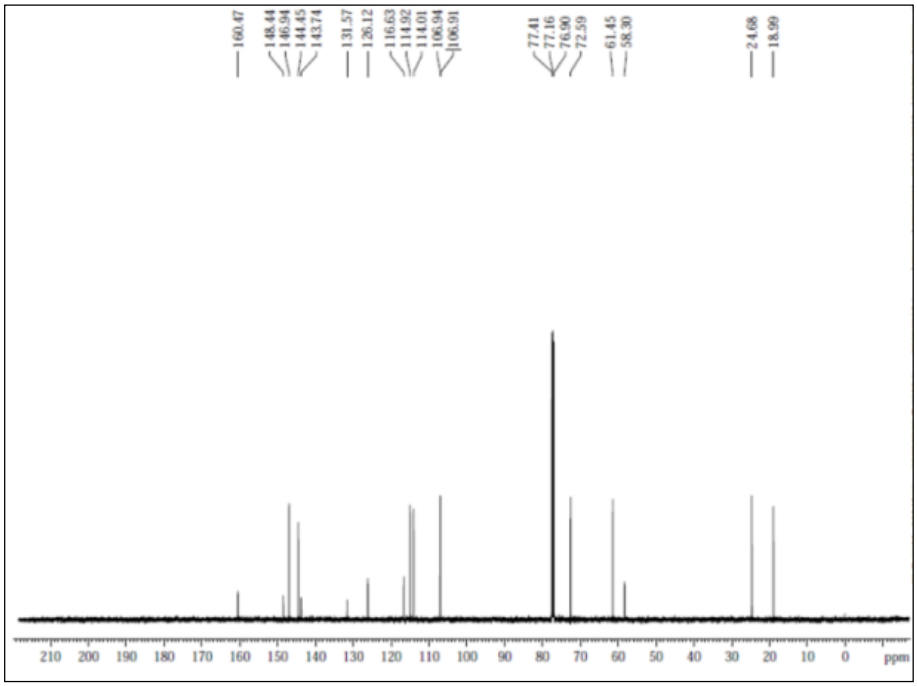

Fig 1(b): 13 C NMR of Heraclenin

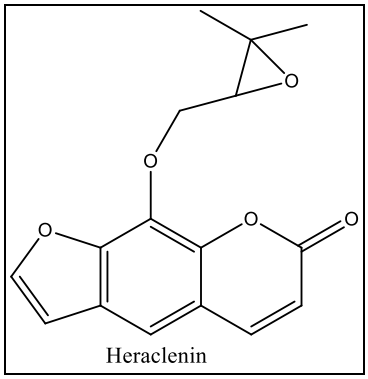

Fig 1(c): Structure of Heraclenin

NMR of osthol

Pale yellow crystals; Yield: 140 mg.

^{1}H NMR: 7.60(d,1H,J=9.2Hz), 7.30(d,1H,J=8.8Hz), 6.84(d,1H,J=8.8Hz), 6.23(d,1H,J=9.2Hz), 5.23(t,1H,J=7.2Hz), 3.54(d,2H,J=7.2Hz), 1.84(s,3H), 1.69(s,3H).

^{13}C NMR: 161.40, 160.24, 152.86, 143.74, 132.67, 126.19, 121.12, 118.04, 113.03, 107.36, 77.33, 77.02, 76.70, 56.05, 25.78, 21.94, 17.93.

According to the literature and comparing the spectrum values (Fig: 2), the compound was identified as osthol [21].

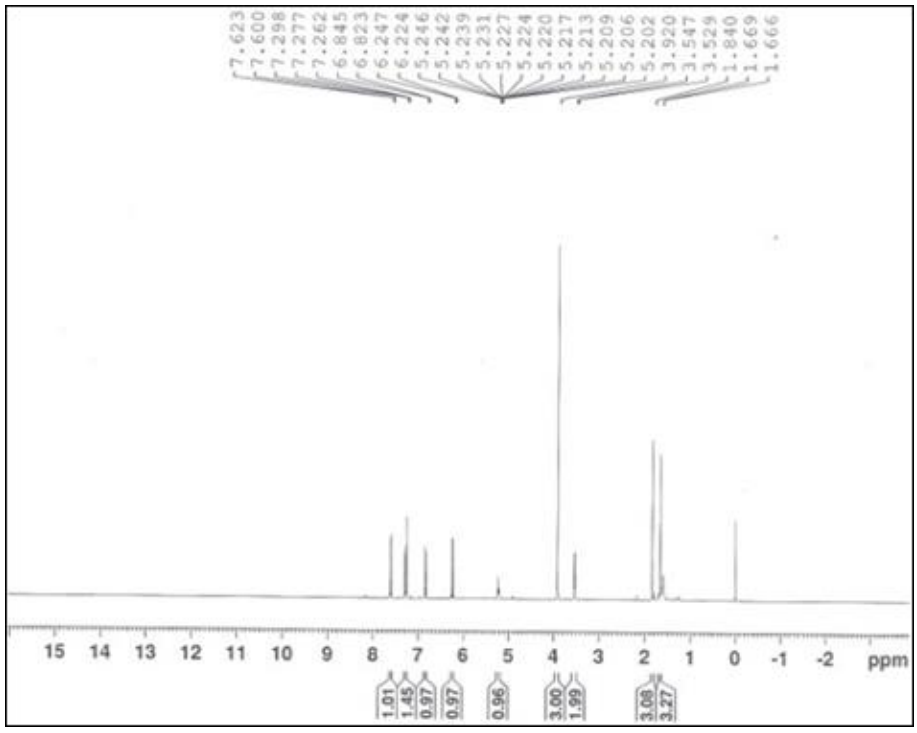

Fig 2(a): 1 H NMR of Osthol

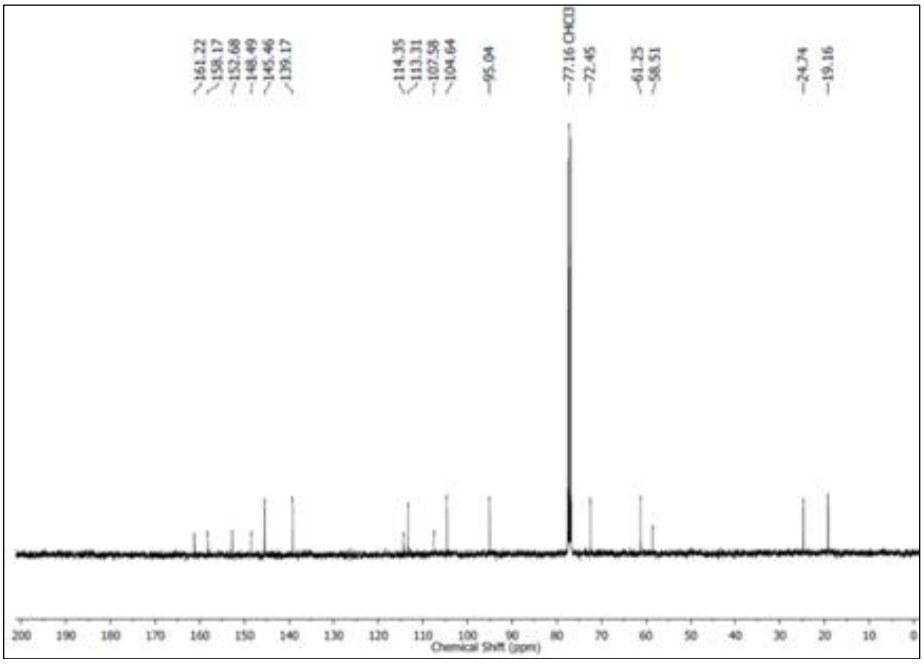

Fig 2(b): 13 C NMR of Osthol

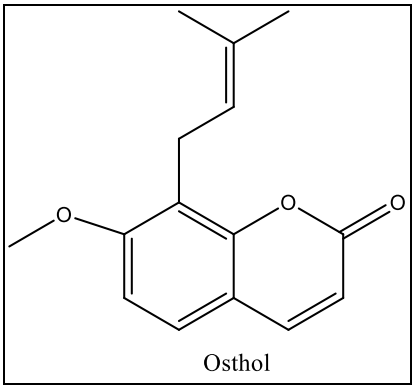

Fig 2a: Structure of Osthol

NMR of oxypeucedanin

White crystals; Yield: 53 mg.

^{1}H NMR: 8.23(d,1H,J=9.9 Hz), 7.62(d,1H,J=2.4 Hz), 7.20(s,1H), 6.96(d,1H,J=2.2 Hz), 6.33(d,1H,J=9.8 Hz), 4.61(dd,1H,J=10.8 Hz,4.3 Hz), 4.46(dd,1H,J=10.5 Hz,6.5 Hz), 3.32(dd,1H,J=4.3 Hz,6.5 Hz).

^{13}C NMR: 161.22, 158.17, 152.68, 148.49, 145.46, 139.17, 114.35, 113.31, 107.58, 104.64, 95.04, 72.45, 61.25, 58.51, 24.74, 19.16. The isolation of oxypeucedanin was confirmed as the NMR spectral data (Fig: 3) was in agreement with the previous studies [22].

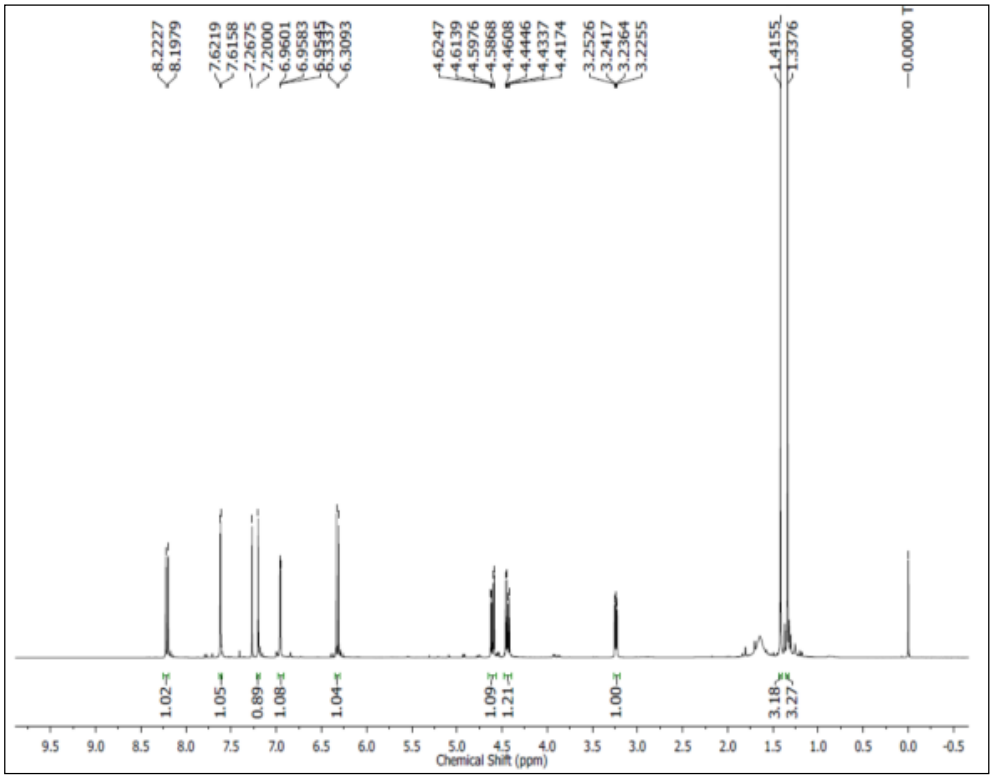

Fig 3(a): 1 H NMR of oxypeucedanin

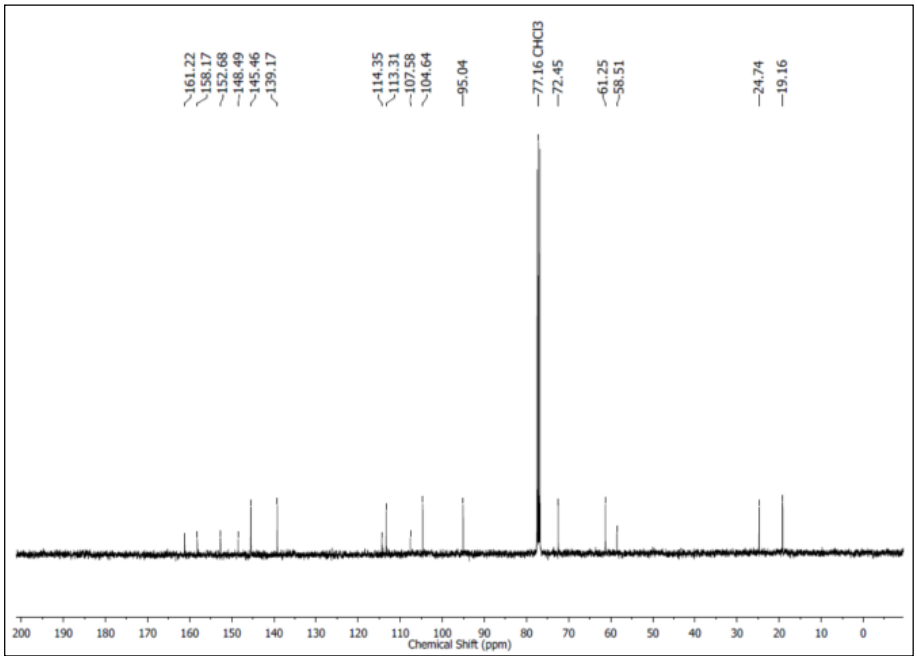

Fig 3(b): 13 C NMR of oxypeucedanin

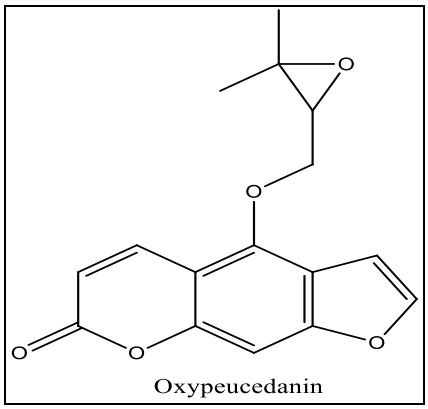

Fig 3(c): structure of oxypeucedanin

Biotransformation of compounds

Heraclenin was bio transformed to a new compound by the reduction process but *Saccharomyces cerevisiae* was not capable of bio transforming the osthol as it doesn't have epoxide ring. So, osthol was chemically transformed to epoxy osthol and thus the *Saccharomyces cerevisiae* was able to reduce it by breaking down the epoxide ring.

NMR of hydroxy imperatorin

Pale yellow; Yield: 69 mg.

^{1}H NMR: 7.78(d,1H,J=9.5 Hz), 7.70(d,1H,J=2.0 Hz), 7.40(s,1H), 6.84(d,1H,J=2.0 Hz), 6.34(d,1H,J=9.5 Hz), 4.76(dd,1H,J=10 Hz,2.5 Hz), 4.34(dd,1H,J=10 Hz,2.5 Hz), 3.84(d,1H,J=8.0 Hz), 3.34(m,1H).

^{13}C NMR: 160.47, 148.44, 146.88, 144.37, 114.84, 113.82, 106.91, 76.04, 75.76, 29.70, 26.69, 25.09.

In the proton NMR of the product (Fig: 4) there is an absence of triplet peak at 3.31 indicating that the reaction has taken place in the epoxide ring of the heraclenin. Further the adjacent peak at 3.8 d and a multiplet at 3,34 indicates the ring opening of the epoxide In 13C NMR there is absence of epoxide peak at 61.45 and 58.30 and the presence of peak at 75.76 and 76.04 indicates the reaction in the epoxide ring. The presence of the additional CH_2 peak at 29.70 and the OH peak at 76.04 clearly indicates that heraclenin been reduced to hydroxy imperatorin.

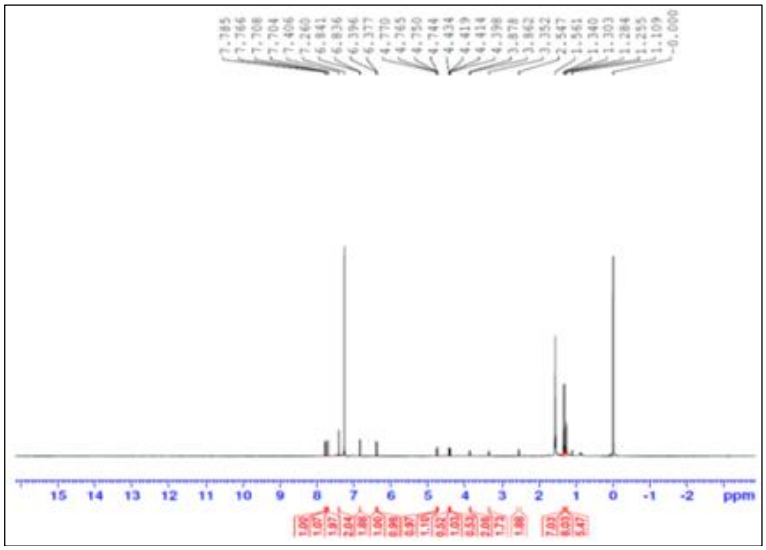

Fig 4(a): 1 H NMR of hydroxy imperatorin

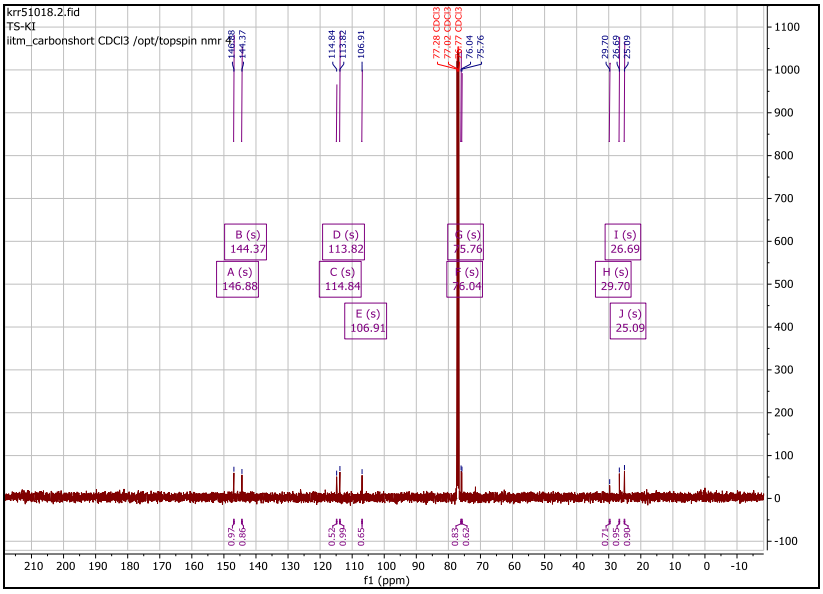

Fig 4(b): 13 C NMR of hydroxy imperatorin

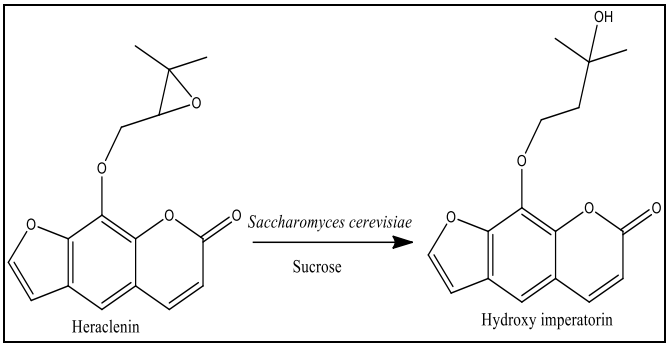

Fig 4(c): Biotransformation of heraclenin

Biotransformation of epoxy osthol

NMR of epoxy osthol

White powder; Yield: 71 mg.

^{1}H NMR: 6.23 (d,1H,J=9.4 Hz), 7.62 (d,1H, J=9.4 Hz), 7.36 (d,1H,J=8.6 Hz), 6.87(d,1H,J=8.6 Hz), 3.02(m,2H), 3.17(dd,1H,J=3 Hz,8.6 Hz), 1.49(s,1H), 1.28(s,1H), 3.93(s,3H).

^{13}C NMR: 160.89, 160.59, 153.27, 143.64, 127.11, 114.01, 112.93, 112.83, 107.32, 62.84, 59.16, 56.03, 24.64, 22.37, 19.01. Comparision of the 1H NMR data of the product epoxy osthol (Fig: 5) with osthol showed that the triplet due to alkenic methine disappeared. Instead, a multiplet appeared at 3.17 ppm corresponding to one proton. The benzylic methylene appeared as a multiplet at 3.02 ppm. The gem dimethy group was separated by 0.2 ppm and clearly appeared as two singlets. In addition, appearance of new signals, a quarternary carbon at 59.1 ppm and a methine at 62.8 ppm in C13 spectrum confirmed the structure to be 7-methoxy-8-(3-methyl-2,3-epoxybutyl) coumarin. This was further supported by DEPT and 1H-13C COSY experiments.

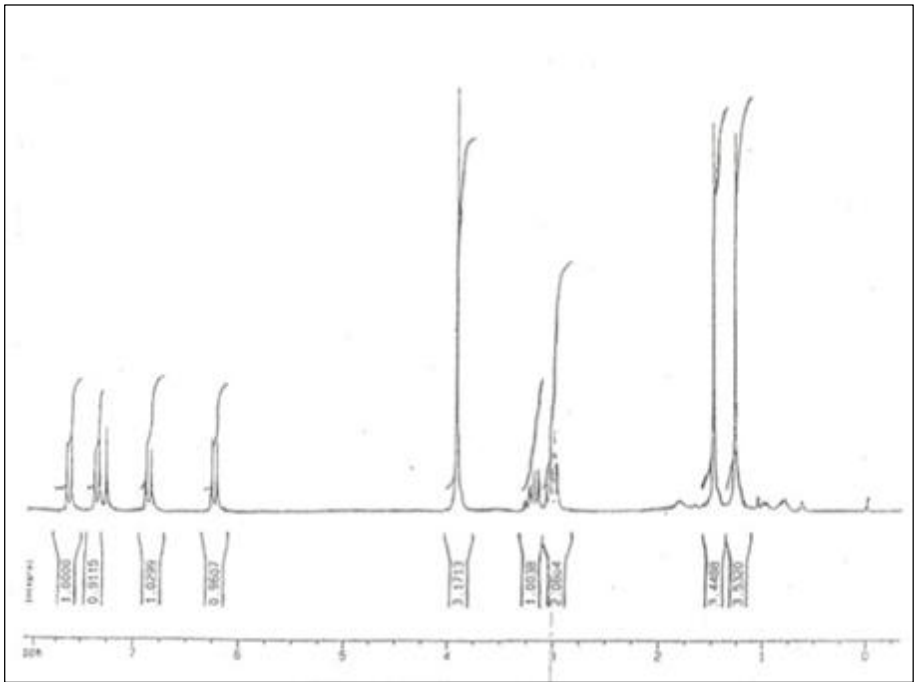

Fig 5(a): 1 H NMR epoxy osthol

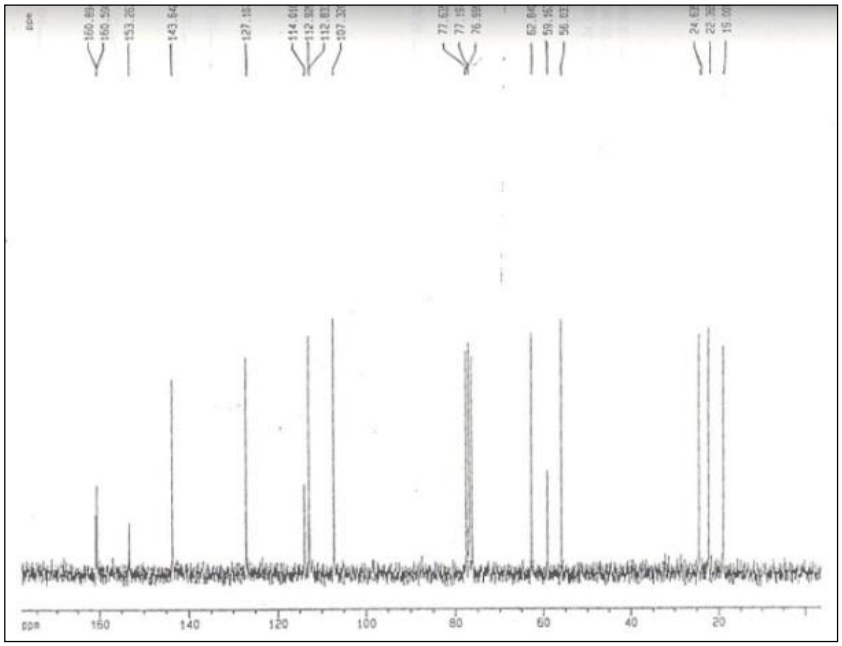

Fig 5(b): 13 C NMR epoxy osthol

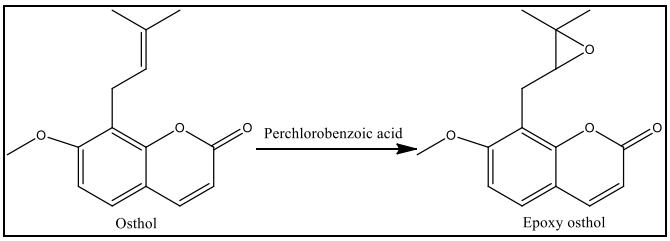

Fig 5(c): Chemical transformation of osthol

NMR of transformed hydroxy osthol

Pale yellow; Yield: 18 mg.

^{1}H NMR: 7.65(d,1H,J=9.2 Hz), 7.36(d,1H,J=8.8 Hz), 6.26(d,1H,J=9.2 Hz), 6.88(d,1H,J=8.8 Hz), 3.94(s,3H), 3.65(dd,1H,J=12.0 Hz,4 Hz), 3.13(dd,1H,J=12.0 Hz,4 Hz), 3.03(dd,1H,J=12.0 Hz, 4 Hz), 2.32(m,1H,J=12.0 Hz), 6.34(s,6H).

^{13}C NMR: 143.97, 127.04, 113.13, 107.44, 78.39, 73.07, 56.28, 29.71, 26.06, 25.28, 23.99.

The ^{1}H NMR of the bio transformed product (Fig: 6) is the same as that of osthol except for changes in the epoxide ring. The absence of doublet of the doublet (dd) at 3.013 and the presence of peak at 3.65, 3.03, 3.13 and 2.32 indicate that epoxide moiety has undergone the reaction. In ^{13}C NMR spectrum there is absence of peak at 62.84 and 59.10. Instead, there is an OH peak at 78.39 indicates that the epoxide ring may be reduced to alcohol and in DEPT 135 in which the additional CH_2 peak at 29.71 and a (qC-OH) peak at 78.38. All the above facts clearly indicate that the epoxy osthol has been reduced to hydroxy osthol.

Fig 6(a): 1 H NMR hydroxy osthol

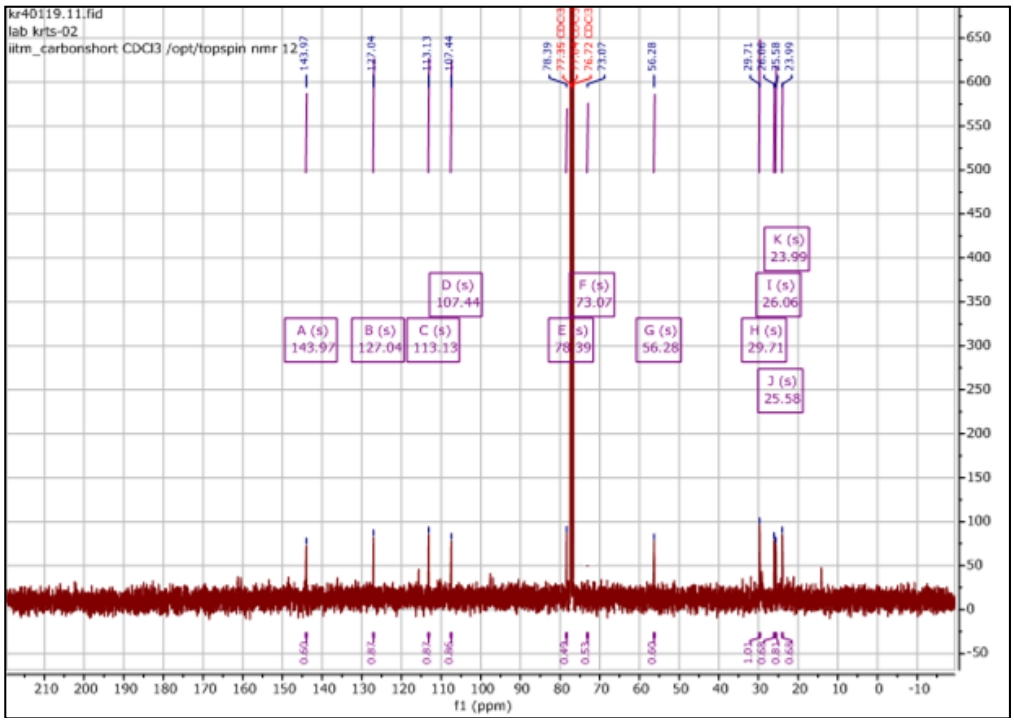

Fig 6(b): 13 C NMR hydroxy osthol

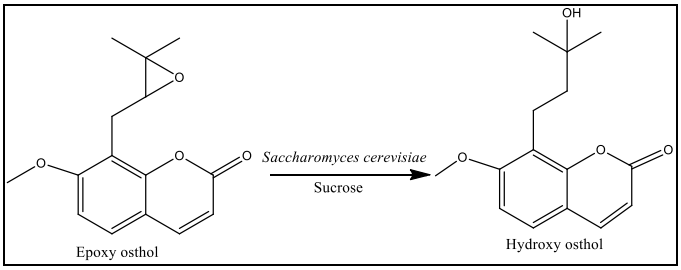

Fig 6(c): Biotransformation of osthol epoxide

Most of the reviewed literature focus on the reduction of the carbonyl compounds (both- simple and conjugated) and there is no much report on the reduction of the secondary metabolites using the simple eco-friendly system. In this research, compounds have been isolated from the non-polar solvent hexane. Three compounds have been isolated and two compounds (heraclenin and osthol) have been transformed by *Saccharomyces cerevisiae* by the reduction of the epoxide ring and not by the reduction of α, β unsaturated double bonds.

Conclusion

Biotransformation has proven to be very useful for the synthesis of new compounds. It makes it possible to derive the compounds without requiring additional steps. Although the yields of the bio transformed products are minimal, the media composition and the extraction protocol can be modified to get the better yield. In the future, these compounds can be further analyzed for their enhanced bioactivity to that of the parent compounds. Having known the potential of biotransformation, more and more compounds can be synthesized and can find its application in the field of agriculture, industry and health sector.

Acknowledgement

The authors gratefully acknowledge the Director, Centre for Plant Molecular Biology and Biotechnology for extending the facilities to carry out the research.

Corresponding author address

Dr. V.P. Santhanakrishnan, Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu - 641301, India. Email ID: santhanakrishnan.vp@tnau.ac.in
, Contact: 9486813794.

References

1. Banday JA, Mir FA, Qurishi MA, Koul S, Razdan TK. Isolation, structural, spectral, and thermal studies of imperatorin micro-crystals from *Prangos pabularia*. Journal of thermal analysis and calorimetry. 2013; 112(3):1165-70.
2. Chopra R, Nayar S, Chopra I. Glossary of Indian medicinal plants, reprinted edition. Publications and Information Directorate, CSIR, New Delhi, 1986, 194.
3. Baer KH, Demirci B, Demirci F, Bedir E, Weyerstahl P, Marschall H *et al*. A new bisabolene derivative from the essential oil of *Prangos uechtritz* fruits. Planta medica. 2000; 66(07):674-7.
4. Ulubelen A, Topcu G, Tan N, Ölçal S, Johansson C, Üçer M *et al*. Biological activities of a Turkish medicinal plant, *Prangos platychlaena*. Journal of ethnopharmacology. 1995; 45(3):193-7.
5. Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzhimatov OK *et al*. Chemical constituents of *Prangos tschimganica*; structure elucidation and absolute configuration of coumarin and furanocoumarin derivatives with anti-HIV activity. Chemical and Pharmaceutical Bulletin. 2001; 49(7):877-80.
6. Tada Y, Shikishima Y, Takaishi Y, Shibata H, Higuti T, Honda G *et al*. Coumarins and γ-pyrone derivatives from *Prangos pabularia*: antibacterial activity and inhibition of cytokine release. Phytochemistry. 2002; 59(6):649-54.
7. Razavi SM, Nazemiyeh H, Delazar A, Hajiboland R, Rahman MM, Gibbons S *et al*. Coumarins from the roots of *Prangos uloptera*. Phytochemistry Letters. 2008; 1(3):159-62.

8. Sadraei H, Shokoohinia Y, Sajjadi S, Ghadirian B. Antispasmodic effect of osthole and *Prangos ferulacea* extract on rat uterus smooth muscle motility. Research in Pharmaceutical Sciences. 2012; 7(3):141.
9. Sadraei H, Shokoohinia Y, Sajjadi S, Mozafari M. Antispasmodic effects of *Prangos ferulacea* acetone extract and its main component osthole on ileum contraction. Research in Pharmaceutical Sciences. 2013; 8(2):137.
10. Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, and Takaishi Y *et al*. Novel antioxidants isolated from plants of the genera Ferula, Inula, Prangos and Rheum collected in Uzbekistan. Phytomedicine. 2004; 11(7-8):645-51.
11. Zahri S, Razavi SM. Cytotoxic effect of *Prangos pabularia* extract on HeLa cell line a medicinal plant. International Journal of Medical Research & Health Sciences. 2016; 5(11):547-52.
12. Tabanca N, Tsikolia M, Ozek G, Ozek T, Ali A, Bernier UR *et al*. The Identification of Suberosin from *Prangos pabularia* Essential Oil and Its Mosquito Activity Against *Aedes aegypti*. Records of Natural Products, 2016, 10(3).
13. Zahri S, Razavi SM, Niri FH, Mohammadi S. Induction of programmed cell death by *Prangos uloptera*, a medicinal plant. Biological research. 2009; 42(4):517-22.
14. Numonov S, Bobakulov K, Numonova M, Sharopov F, Setzer WN, Khalilov Q *et al*. New coumarin from the roots of *Prangos pabularia*. Natural product research. 2018; 32(19):2325-32.
15. Chandra R, Kumar V. Biotransformation and Biodegradation of Organophosphates and Organohalides. Environmental Waste Management: CRC Press; 2016; 489-538.
16. Chin-Joe I, Straathof AJ, Pronk JT, Jongejan JA, Heijnen JJ. Influence of the ethanol and glucose supply rate on the rate and enantioselectivity of 3-oxo ester reduction by baker's yeast. Biotechnology and bioengineering. 2001; 75(1):29-38.
17. Nestl BM, Nebel BA, Hauer B. Recent progress in industrial bio catalysis. Current opinion in chemical biology. 2011; 15(2):187-93.
18. Simeo Y, Sinisterra J. Biotransformation of terpenoids: a green alternative for producing molecules with pharmacological activity. Mini-Reviews in Organic Chemistry. 2009; 6(2):128-34.
19. Ema T, Sugiyama Y, Fukumoto M, Moriya H, Cui J-N, Sakai T *et al*. Highly enantio selective reduction of carbonyl compounds using a reductase purified from bakers' yeast. The Journal of Organic Chemistry. 1998; 63(15):4996-5000.
20. Razdan T, Kachroo V, Harkar S, Koul G. Furanocoumarins from *Heracleum canescens*. Phytochemistry. 1982; 21(4):923-7.
21. Zhang L, Sun A, Li A, Kang J, Wang Y, Liu R. Isolation and purification of osthole and imperatorin from Fructus Cnidii by semi-preparative supercritical fluid chromatography. Journal of Liquid Chromatography & Related Technologies. 2017; 40(8):407-14.
22. Wei Y, Ito Y. Preparative isolation of imperatorin, oxypeucedanin and isoimperatorin from traditional Chinese herb “bai zhi” *Angelica dahurica* (Fisch. ex Hoffm) Benth. ET Hook using multidimensional high-speed counter-current chromatography. Journal of Chromatography A. 2006; 1115(1-2):112-7.