Supplementary material

Fatty acid analysis by GC/MS

Briefly, for 100 mg of the oil sample, 1 mL of Boron trifluoride (BF_3) in methanol was added and kept in water bath for 30 min at 60 °C. The tubes were immediately transferred into ice bath for 5 min and 1 mL hexane was added followed by 1 mL distilled water and the tubes were vortexed for complete mixing. The reaction mixture tubes were kept aside for layer separation and the upper layer was collected in tube containing anhydrous sodium sulfate for removal of moisture. Finally, the undisturbed top methyl ester layer free of water and residual particles were transferred to GC vials for GC/MS analyses (Agilent technologies, Milan, Italy).[18]

GC/MS analysis was performed by using an Agilent HP-7890B chromatograph connected directly to a 5977 inert mass spectrometer (Agilent technologies, Milan, Italy), with GC column, DB-23 (60m 0.25mm I.D 0.25mm film thickness). The analyses were performed in split less mode (0.5 min), inlet temperature was 250 °C, and the carrier gas Helium at a flow rate of 1 mL/min was used. The temperature was programmed at 10 °C/min to 300 °C, and then isothermal at 300 °C for 5 min. The MS detector was operated in electron ionization (EI) mode (70eV, 200mA), in full-scan mode (m/z 40-400) and in selected–ion monitoring (SIM) mode (ions at m/z 127, 140 and 256 for heptadeconoic acid as the internal standard). The transfer line was set at 290 °C and the solvent delay was set at 3 min.

Determination of total phenolics content (TPC)

Briefly, different concentrations of sample extracts to fit into standard concentrations were taken, 1.0 mL of 10% Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate (Na_2CO_3) were added, vortexed thoroughly and incubated at room temperature in darkness for 90 min and the absorbance was read at 725 nm using double beam spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan).[22]

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Briefly, 100 – 400 µL of sample extract or standard (50 – 150 µL) was added to a same volume of DPPH reagent (0.1 mM in methanol) to a final reaction mixture of 2 mL and vortexed vigorously. The reaction tubes were incubated in dark for 20 min, and the discoloration of DPPH was measured at room temperature against a reagent blank at 517 nm using a double beam spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan).[30]

Ferric reducing antioxidant power (FRAP)

This method is based on the ability of the sample to reduce Fe^{+3} to Fe^{+2} ions. In the presence of 2,4,6-tripyridyl-striazine (TPTZ), the Fe^{+2}-TPTZ complex exhibits blue colour which has absorption maxima at 593 nm. Briefly, to different concentrations of oil extracts, 3.0 mL of working FRAP reagent was added. After incubation for 6 min at room temperature the absorbance was measured at 593 nm against Trolax as standard and the results are expressed as mM of Trolox/100 g of oil.[31]

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging activity assay

For this assay, 13.2 mg potassium persulphate was mixed with 20 mL of 7.4 mM ABTS solution (in water) for about 16 h at room temperature in the dark to yield a dark blue–green solution. The solution was diluted with PBS (pH 7.4) to attain an absorbance value of 0.70 at 734 nm, and used for the antioxidant assay within 4 h. The sample extractives at different concentrations were mixed with 2 mL of the diluted ABTS solution for 6 min, followed by measurement of light absorbance at 734 nm.[32]

Separation of phenolics and flavonoids by HPLC

A binary solvent system, consisting of filtered Milli-Q water adjusted to pH 2.6 with acetic acid as solvent A and 80% acetonitrile as solvent B, was run for 60 min at a flow rate of 1.2 mL/min. The phenolics and flavonoids were detected at 280 and 320 nm with the help of standards.[33]

Column purification of squalene

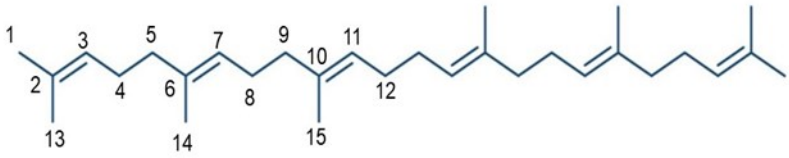
A 0.5 g of USM was dissolved in 5 mL of petroleum ether and added 1 g of silica and mixed thoroughly. Later the mixture was rotary evaporated to remove the organic solution until dryness. The left over residual silica powder coated with unsaponifiables was loaded to silica column and the compound was eluted with petroleum ether, at a flow rate of approximately 1.0 mL/min. Test tubes (10 mL with screw cap) were used for fraction collecting. Thin-layer chromatography (TLC) was simultaneously performed to detect the squalene compound detection in the fractions with petroleum ether-ethyl acetate (6:1, v/v) and the spots were visualized by phosphomolybdic acid. Squalene eluted completely in the initial fractions (fraction 1 to fraction 8) with petroleum ether eluate. The eluates were independently concentrated under vacuum to give squalene fraction. The residues in the column were washed-out by methanol.

Supplementary Tables

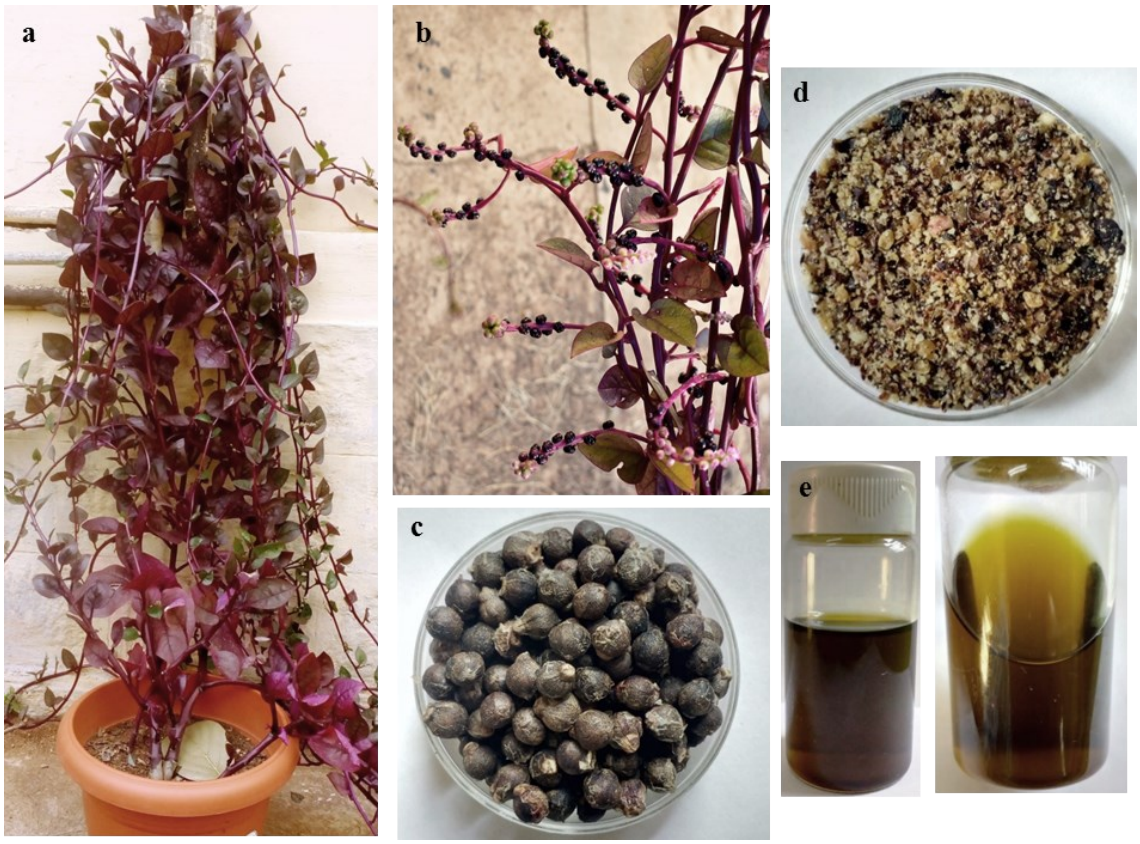
Supplementary Table 1 Extracted ^{1}H and ^{13}C-chemical shift in ppm. Signals from spectrum have been assigned hydrogen and carbon atom groups (1 through 15) from the structure shown at bottom.

Atom marked	δ ^{1}H-NMR (ppm)	δ ^{13}C-NMR (ppm)
1	1.60	24.67
2	5.0-5.17	130.24
3	5.0-5.17	123.38
4	2.07	27.25
5	1.98	38.73
6	5.0-5.17	134.09
7	5.0-5.17	123.28
8	2.0	25.74
9	1.98	38.71
10	5.0-5.17	133.88
11	5.0-5.17	123.25
12	2.07	25.64
13	1.60	16.66
14	1.68	15.02
15	1.68	14.98

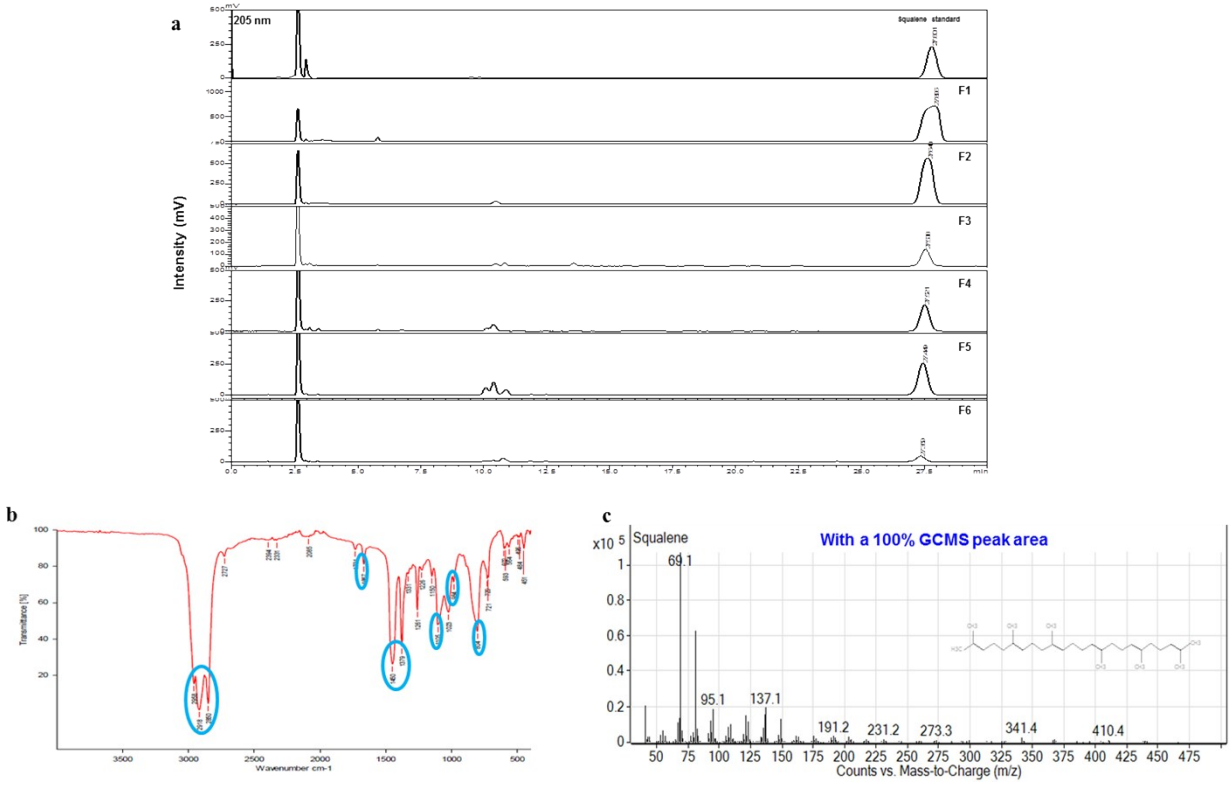
Structure of squalene



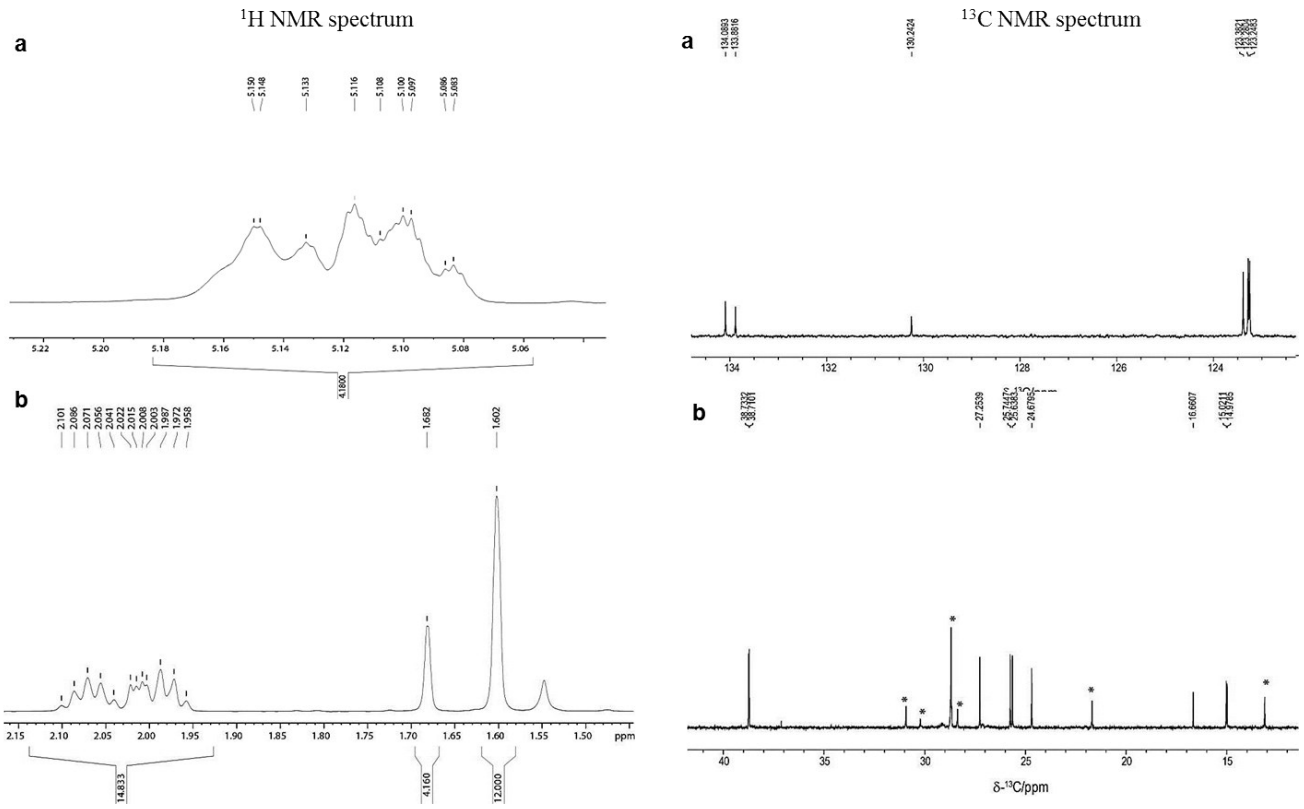
Supplementary Figures



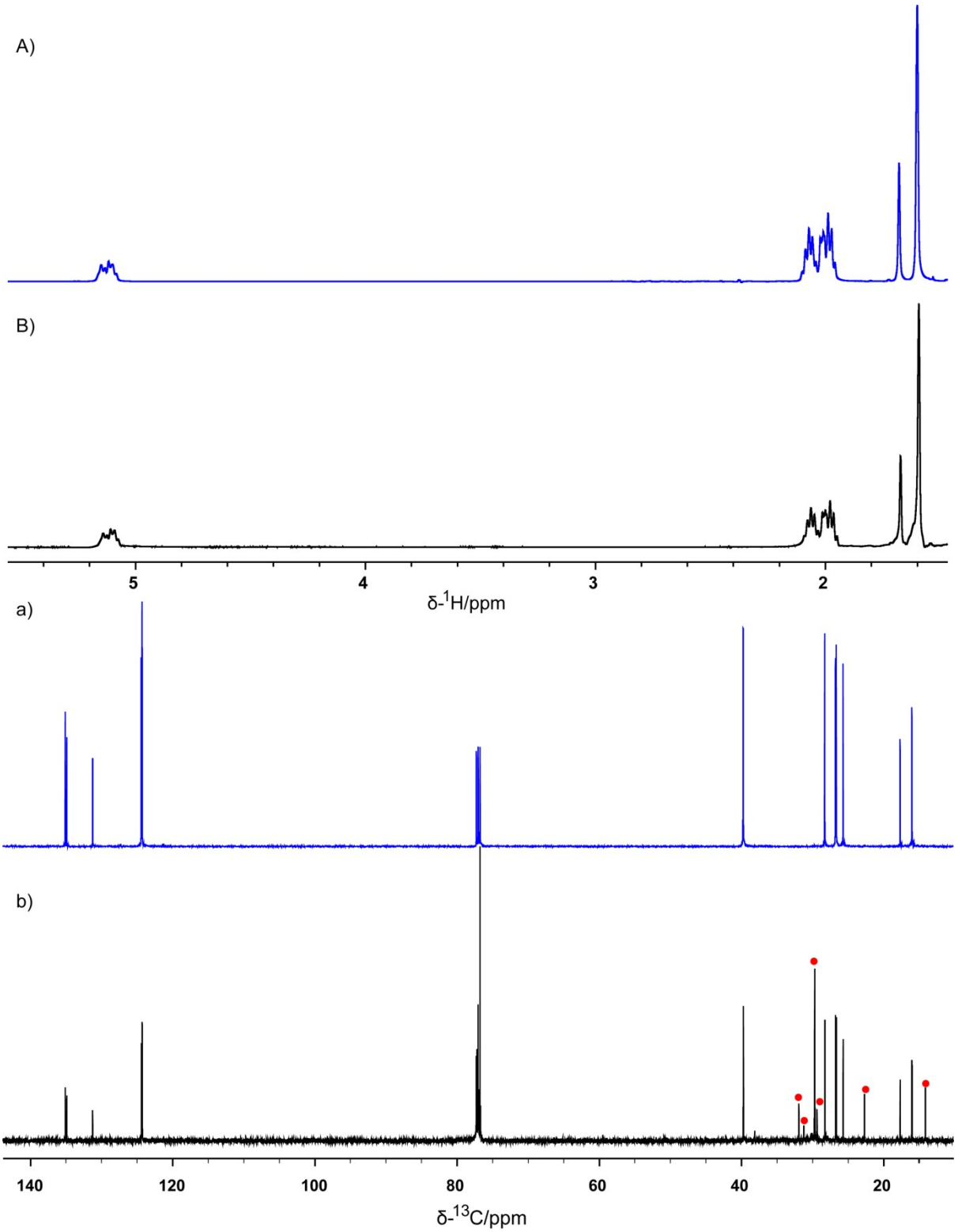
Supplementary Fig. 1 Images representing the twining plant of *B. rubra* (a), the inflorescence containing fruits (b), collected seeds after removing pulp (c), seed powder prepared for oil extraction (d), and oil extracted (e).



Supplementary Fig. 2 a. HPLC separation profile, b. FTIR finger printing and c. GCMS confirmation of *B. rubra* seed oil purified squalene fraction.



Supplementary Fig. 3 Expanded ^{1}H NMR spectrum (a and b) left trace and ^{13}C NMR spectrum (a and b) right trace of an extracted squalene compound dissolved in $CDCl_3$ solvent. Asterisks (*) denote the residual solvent impurity (n-hexane).



Supplementary Fig. 4 Stack plot of ^{1}H and ^{13}C-NMR spectrum of squalene standard (blue colour) and purified squalene (black colour). Upper trace shows the ^{1}H-NMR and lower trace shows $^{13}C\{^{1}H\}$-NMR spectra. The red colour dot indicates the solvent traces impurity peaks.