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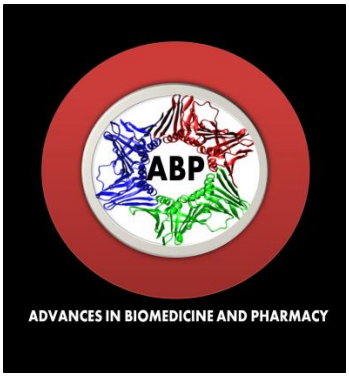

Original Article

Chemical constituents of *Salvia sclarea* from Kashmir (India).

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Abstract

Salvia sclarea (Lamiaceae) commonly known as clarysage, is a medicinally important herb showing broad range of biological activities. This plant is mainly known because of its pleasantly smelling essential oils rich in linalool and linalyl acetate. In addition to linalool and linalyl acetate (major essential oil secondary metabolites), the peculiar aromatic note of the essential oil is because of the minor constituents including α-terpineol, geranyl acetate, (E)-caryophyllene, limonene, thymol, nerol, geranyl acetate etc. Phytochemical investigation of the aerial parts of this plant yielded nine compounds i.e. β-sitosterol, α-amyrin, ursolic acid, oleanolic acid, betulinic acid, sclareol, salvigenin, acacetin and norartocarpetin. The structures of these compounds have been established by spectroscopic methods (UV, IR, ^{1}H NMR, ^{13}C NMR and MS) in light of literature. Structure of ursolic acid has also been established by HMBC experiments. Ursolic acid, oleanolic acid, betulinic acid, salvigenin, acacetin and norartocarpetin are reported for the first time from this plant.

Keywords: *Salvia sclarea*, triterpenes, flavones, sclareol, salvigenin, NMR.

Introduction

Salvia (commonly known as sage), the largest genus of the family Lamiaceae, represents an enormous and cosmopolitan assemblage of nearly 1000 species displaying a remarkable range of variation. The genus comprises 500 species in Central and South America, 250 species in Central Asia/Mediterranean and 90 species in Eastern Asia [1]. Because they readily cross-pollinate, innumerable hybrids, both natural and manmade, are also found. India is a major diversity centre for most members of Lamiaceae in Asia [2]. *Salvia* is a fascinating plant genus, it features prominently in the pharmacopoeias of many countries throughout the world. *Salvia* species have been used in many ways, e.g. essential oils used in perfumery, the flowers used as rouge, the leaves used for varicose veins, the seed oil as an emollient, and the roots as a tranquiliser. The range of traditional applications of the herb in domestic medicine seems to be endless: it has been used as a medication against perspiration and fever; as a carminative; a spasmolytic; an antiseptic/bactericidal; an astringent; as a gargle or mouthwash against the inflammation of the mouth, tongue and throat; a wound-healing agent; in skin and hair care; and against rheumatism and sexual debility in treating mental and nervous conditions as well as an insecticidal [3]. Some species of *Salvia* have been cultivated worldwide for use in

folk medicine, in flavour and fragrance industry and for culinary purposes [4].

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. *Salvia* species are important group of useful plants which have not lost their medicinal importance since ancient times. An Anglo-Saxon manuscript reads "Why should a Man die, if sage grows in his garden?" [3]. This reflects the medicinal importance of *Salvia* species since the existence of ethnopharmacology. *Salvia*, commonly known as sage, has multiple uses such as condiment, food additive, spice and herbal tea [5]. The seeds of *Salvia* species often produce mucilage on wetting which is used to produce pleasant drinks and in the treatment of eye diseases [6, 7]. In addition to this, *Salvia* species have been used for memory-enhancing purposes in European folk medicine [8, 9].

Genus *Salvia* has attracted great interest primarily because of its pharmacological potential and has been the subject of numerous chemical studies. The main secondary metabolite constituents of *Salvia* species are terpenoids and flavonoids. The aerial parts of these plants contain flavonoids, triterpenoids, and monoterpenes, particularly in the flowers and leaves, while diterpenoids are found mostly in the roots. However, literature reveals that some American *Salvia* species also contain diterpenoids in the aerial parts, and in certain *Salvia* species, triterpenoids and flavones are present in the roots [10]. Various species of the genus *Salvia* are well known throughout the world for their ethnopharmacological properties which have made it an attractive choice for many researchers. Some *Salvia* species have been scientifically studied in many parts of the world and are reported to have various biological activities including antibacterial [11], antioxidant [12], anti-inflammatory (Perry *et al*., 2003, anticancer [13] and anticholinesterase [8].

Salvia species are considered as a rich source of polyphenolics and flavonoids. More than 160 polyphenols have been identified, some of which are unique to the genus. A large number of these polyphenolic compounds are apparently constructed from the caffeic acid building block via a variety of condensation reactions [4]. The polar phenolic acids supposedly constitute the major part of the *Salvia* decoction. Caffeic acid plays a central role in the biochemistry of Lamiaceae and occurs predominantly in the dimer form as rosmarinic acid [14]. In *Salvia* species, caffeic acid is the building block of a variety of the plant metabolites from the simple monomers to multiple condensation products that give rise to a variety of oligomers [4]. Phenolic acids such as rosmarinic, caffeic and salvimanolic acids have been isolated [4]. The phenolic glycosides are not very common in *Salvia*. The rosmarinic acid 3'-glucoside and its methyl ester, as well as the *cis*- and *trans*-*p*-coumaric acid 4-*O*-(2-*O*-apyiosyl) glucosides, are the only examples of the glycosylated phenolic acids [4]. In contrast, flavonoids are widely distributed in *Salvia* species [15] and they are mostly present as flavones, flavonols and their glycosides. The majority of *O*-flavonoids are the flavones, apigenin and luteolin. The 6-hydroxy flavones are the flavonoids that characterise the species of *Salvia* and they include a variety of 6-hydroxylated apigenin and luteolin derivatives with cirsimaritin. Flavonols are mostly those of kaempferol and quercetin. Flavonone *O*-glycosides are apparently common in *Salvia* and most of them are flavone 7-glucosides represented by cosmosiin and cinaroside [4].

The review of literature indicated that the genus *Salvia* has been a popular topic in phytochemical and ethnobotanical research. The solvent extracts, essential oils and compounds isolated from various *Salvia* species unveiled their broad range of pharmacological properties, both *in vitro* and *in vivo*.

Salvia sclarea commonly known as clary sage, has vast traditional uses. The herb is antispasmodic and balsamic in nature and has been used for digestive difficulties as a stomachic. It has also been employed in treatment of kidney disease with good results [16]. Mucilage of the seeds is used in tumours [17]. Cold extract of clary sage have been used to draw out thorns and splinters and reduce inflammation. The powdered roots have been used as snuff to clear the head and ease a headache [18]. This herb has been used by the native Jamaicans, for cooling and cleansing of ulcers, and also for the treatment of inflammation of the eyes. A decoction of the leaves boiled in coconut oil has been considered beneficial for the stings of scorpions [19].

The essential oil from *S. sclarea* has come under increasing attention for its use in aromatherapy. It is said to act on the brain's thalamus which helps to relieve anxiety states, including those involving fear, paranoia and delusions [3]. It is considered as a good relaxing oil with euphoric effect on sensitive people and may help in the treatment of insomnia [20]. The oil has also been used for its antidepressant, antiseptic, antispasmodic, astringent, carminative and deodorant properties [21]. Because of the non-toxic nature, the essential oil has been used as a fragrance component and a flavouring in the food industry [3]. The essential oil has shown *in vivo* antidepressant-like effects by modulation of the DAnergic pathway [22].

Previous phytochemical studies on acetone extracts of the whole plant of *S. sclarea* from Turkey have resulted in the isolation of sixteen diterpenes, sclareol, manool, salvipisone, ferruginol, microstegiol, candidissiol, 7-

oxoroyleanone, 2,3-dehydrosalvipisone, 7-oxoferruginol-18-al, aethiopinone, 1-oxoaethiopinone, salvinolone, cryptojaponol, δ^7-manool, sclareapinone and acetylsalvipisone; two sesquiterpenes, caryophyllene oxide and spathulenol; and the flavonoids; apigenin, luteolin, 4′-methylapigenin, 6-hydroxyluteolin-6,7,3′,4′-tetramethyl ether, 6-hydroxy apigenin-7,4′-dimethyl ether along with α-amyrin, 3-oxo-oleanolic acid and β-sitosterol. The diterpenoids and the sesquiterpenoids were shown to have antimicrobial activity against standard bacterial strains and fungal strains [23, 24]. Recently two amphilectane diterpenes, salviatriene A and salviatriene B have been isolated from n-hexane extract of the full bloom stage calyx of *S. sclarea*. These diterpene molecules are considered as the first representatives of this family to be described from the plant kingdom [25].

The current research programme was undertaken as part of our on-going research programme based on bioprospection of natural products [26-32]. Thorough review of literature revealed no reports of phytochemistry on S. sclarea of Kashmir origin. This encouraged us to undertake the further phytochemical investigation of this plant species. Repeated column chromatography on silica gel and recrystallization techniques of hexane and acetone extract of the floral parts led to the isolation of nine natural compounds (Figure 1).

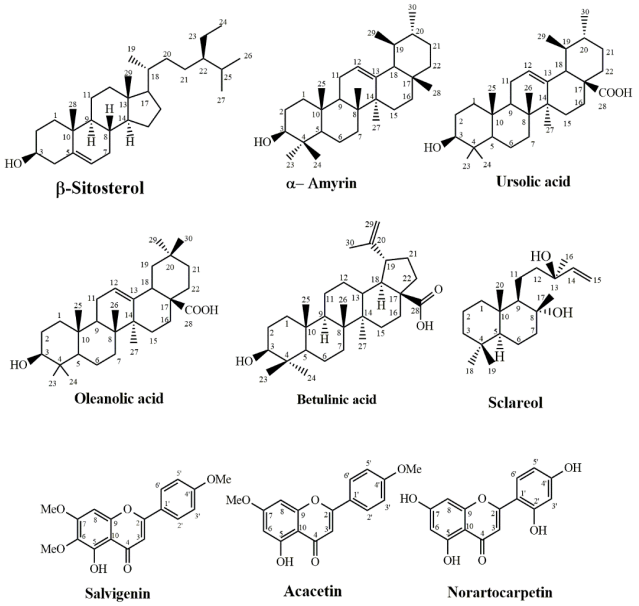

Figure 1. Molecular structures of the isolated compounds.

Materials and Methods

General experimental procedures

^{1}H NMR spectra were recorded as δ values on 500 MHz NMR and ^{13}C NMR on 125 MHz using deuterated $CDCl_3$/pyridine/DMSO as a solvent and TMS as internal standard. Infrared spectra were recorded as KBr pellets in cm^{-1} on a Hitachi 270-30 spectrophotometer. Melting points were determined on a Buchi melting point apparatus. UV spectra were scanned in methanol on Specord S100. HR-EIMS were recorded on an Agilent Technologies G6540-UHD LC/MS Q-TOF. Column was run using silica gel (60-120 mesh). TLC plates were visualized under UV light and after exposure to iodine vapour in iodine chamber.

Plant Material

The aerial parts of *S. sclarea* at different developmental stages were collected from the IIIM gene bank (Srinagar). After proper identification, a voucher specimen (No. 2101/12) was deposited in the Herbarium of the Indian Institute of Integrative Medicine, Srinagar, India.

Extraction and Isolation

Air-dried and coarsely powdered plant material (aerial part, 1.0 Kg) was extracted with hexane for 48 hours. The marc was dried and extracted with acetone for 48 hours. The hexane and acetone extracts thus obtained were concentrated under reduced pressure to give 76.0 g of hexane and 21.0 g of acetone extracts. The extracts thus obtained was dissolved in minimum amount of respective solvents and adsorbed on silica gel to form slurry. The dried slurry of hexane extract was subjected to silica gel column chromatography leading to Fr-I (5% ethyl acetate in petroleum ether), Fr-II (10% ethyl acetate in petroleum ether) and Fr-III (50% ethyl acetate in petroleum ether). Repeated column chromatography of these fractions and acetone extract using different percentages of petroleum ether-ethyl acetate afforded seven compounds from hexane extract viz. β-Sitosterol (**SS-1**), α-Amyrin (**SS-2**), Ursolic acid (**SS-3**), Oleanolic acid (**SS-4**), Sclareol (**SS-6**), Salvigenin (SS-7) and Acacetin (**SS-8**); and two compounds from acetone extract viz. Betulinic acid (**SS-5**) and Norartocarpetin (**SS-9**).

SS-1 (β-Sitosterol)

Colourless crystalline solid; yield**:** 172 mg, mp. 137-139 °C; $[\alpha]_D^{25}$**:** -39° (c 2.0, $CHCl_3$) IR (KBr) $\nu_{max}cm^{-1}$: 3440 (OH), 3060 (C-H), 1655, 815 (C=C); EIMS *m/z*: 414, 399, 396, 381, 329, 303, 273, 255, 83, 69: HR-EIMS: 414.3837 (calculated for $C_{29}H_{50}O$, 414.3861); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.40 (1H, t, J = 3.1 Hz, H-6), 3.42 (1H, dd, J = 11.4, 4.5 Hz, H-3), 2.07 (2H, m, H-2), 0.97 (3H, s, H-19), 0.90 (3H, d, J = 6.5 Hz, H-21), 0.87 (6H, d, J = 5.0 Hz, H-26, H-27) 0.80 (3H, t, J = 7.0 Hz, H-29), 0.69 (3H, s, H-18); ^{13}C NMR($CDCl_3$, 125 MHz) δ: 142.0 (C-5), 121.71 (C-6), 71.82 (C-3), 56.90 (C-17), 56.07 (C-14), 50.17 (C-9), 43.8 (C-4), 43.0 (C-13), 40.80 (C-12),

39.3 (C-22), 38.7 (C-1), 37.1 (C-10), 36.19 (C-20), 33.01 C-7), 31.95 (C-8), 29.9 (C-2), 29.7 (C-16), 29.5 (C-23), 29.2 (C-24), 25.8 (C-15), 23.7 (C-28), 23.11 (C-25), 21.5 (C-11), 21.27 (C-27), 19.82 (C-21), 19.44 (C-19), 18.03 (C-26), 12.24 (C-18), 12.1 (29).

SS-2 (α-Amyrin)

Colourless crystalline solid; yield: 18 mg, mp. 179-183 ^{o}C; IR (KBr) ν_{max}cm^{-1} : 3320 (OH), 1640, 1380, 1372.HR-EIMS: 426.3848 (calculated for $C_{30}H_{50}O$, 426.3862). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.53 (1H, t, J = 3.3 Hz, H-12), 3.85 (1H, d, J = 11.2 Hz, H-3), 1.91 (2H, m, H-11), 1.81 (1H, d, J = 11.8 Hz, H-18), 1.78 (2H, d, J = 3.5 Hz, H-2), 1.58 (1H, m, H-9), 1.52 (2H, m, H-16), 1.51 (2H, m, H-7), 1.48 (2H, d, J = 3.5 Hz, H-1), 1.41 (1H, m, H-19), 1.32 (2H, d, J = 7.3 Hz, H-6), 1.27 (2H, m, H-15), 1.25 (3H, s, H-23), 1.16 (3H, s, H-27), 1.08 (3H, s, H-26), 1.03 (2H, d, J = 10.2 Hz, H-21), 0.95 (3H, s, H-25), 0.94 (2H, d, J = 10.2 Hz, H-22), 0.93 (1H, m, H-20), 0.89 (3H, m, H-28), 0.88 (3H, d, J = 6.2 Hz, H-29), 0.88 (3H, d, J = 6.2 Hz, H-29), 0.82 (1H, m, H-5), 0.80 (3H, s, H-24): ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 139.6 (C-13), 124.5 (C-12), 78.9 (C-3), 59.1 (C-18), 55.3 (C-5), 47.8 (C-9), 42.1 (C-14), 41.5 (C-22), 40.1 (C-8), 39.7 (C-19), 39.6 (C-20), 38.9 (C-1), 38.7 (C-4), 36.9 (C-10), 33.7 (C-17), 33.0 (C-7), 31.2 (C-21), 28.7 (C-28), 28.1 (C-23), 28.1 (C-16), 27.3 (C-2), 26.7 (C-15), 23.4(C-11), 23.2 (C-27), 21.3 (C-30), 18.4 (C-6), 17.4 (C-29), 16.9 (C-26), 15.6 (C-24), 15.6 (C-25).

SS-3 (Ursolic acid)

Colourless crystalline solid; yield: 65 mg; mp. 282-286 ^{o}C; $[\alpha]_D^{25}$: +65° (c 2.0, $CHCl_3$); IR (KBr) ν_{max}cm^{-1}: 3440 (OH), 1700 (C=O), 1640, 1380, 1372; HR-EIMS: 456.3594 (calculated for $C_{30}H_{48}O_3$, 456.3603). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.50 (1H, t, J = 3.8 Hz, H-12), 3.46 (1H, dd, J = 6.4, 9.6 Hz, H-3), 2.62 (1H, m, H-18), 2.30 (1H, m, H-15β), 2.13 (1H, m, H-16α), 2.01 (1H, m, H-16β), 1.95 (2H, d, J = 10.5 Hz, H-22), 1.94 (2H, m, H-11), 1.82 (2H, m, H-2), 1.62 (1H, m, H-9), 1.58 (1H, m, H-7α), 1.58 (1H, m, H-6α), 1.57 (1H, t, J = 3.8 Hz, H-1β), 1.48 (1H, m, H-21β), 1.47 (1H, m, H-19), 1.40 (1H, d, J = 10.5 Hz, H-21α), 1.39 (1H, m, H-6β), 1.37 (1H, m, H-7β), 1.23 (3H, s, H-23), 1.21 (3H, s, H-27), 1.20 (1H, m, H-15α), 1.04 (3H, s, H-26), 1.03 (1H, m, H-20), 1.01 (3H, s, H-24), 1.00 (1H, m, H-1α), 0.98 (3H, d, J = 6.6 Hz, H-29), 0.93 (3H, d, J = 6.6 Hz, H-30), 0.88 (1H, m, H-5), 0.87 (3H, s, H-25); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 179.8 (C-28), 139.3 (C-13), 125.7 (C-12), 79.0 (C-3), 53.6 (C-18), 52.8 (C-5), 48.1 (C-17), 47.6 (C-9), 42.5 (C-14), 40.0 (C-8), 39.5 (C-4), 38.7 (C-1), 37.5 (C-19), 37.5 (C-22), 36.8 (C-10), 36.4(C-20), 34.3 (C-21), 33.3 (C-7), 28.7 (C-15), 24.9 (C-16), 23.9 (C-27), 23.8 (C-23), 23.6 (C-11), 23.5 (C-2), 23.4 (C-30), 21.1 (C-29), 18.8 (C-6), 16.5 (C-24), 16.5 (C-26), 15.7 (C-25).

SS-4 (Oleanolic acid)

Colourless needles; yield: 78 mg; mp. 307-311 ^{o}C; $[\alpha]_D^{25}$: +76.2° (c 2.0, $CHCl_3$); IR (KBr) ν_{max}cm^{-1} : 3450 (OH), 1700 (C=O), 1660, 1382, 1378, 820; EI-HRMS: 456.3611 (calculated for $C_{30}H_{48}O_3$, 456.3603); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.48 (1H, d, J = 3.4 Hz, H-12), 3.58 (1H, dd, J = 6.8, 9.4 Hz, H-3), 3.30 (1H, dd, J = 13.5, 4.2 Hz, H-18), 2.19 (1H, m, H-15β), 2.12 (1H, m, H-16α), 2.04 (1H, m, H-22β), 1.97 (2H, m, H-11), 1.96 (1H, m, H-16β), 1.83 (1H, m, H-19α), 1.82 (2H, m, H-2), 1.81 (1H, m, H-22α), 1.70 (1H, m, H-9), 1.58 (1H, m, H-6α), 1.56 (1H, m, H-1β), 1.53 (1H, m, H-7α), 1.46 (1H, m, H-21α), 1.39 (1H, m, H-6β), 1.36 (1H, m, H-7β), 1.32 (1H, m, H-19β), 1.30 (3H, s, H-27), 1.24 (3H, s, H-23), 1.23 (1H, m, H-21β), 1.22 (1H, m, H-15α), 1.04 (3H, s, H-26), 1.03 (1H, m, H-1α), 1.02 (3H, s, H-30), 1.02 (3H, s, H-24), 0.97 (3H, s, H-29), 0.93 (3H, s, H-25), 0.88 (1H, m, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 180.4 (C-28), 144.1 (C-13), 122.4 (C-12), 78.5 (C-3), 55.5 (C-5), 48.1 (C-9), 46.7 (C-17), 46.1 (C-19), 42.0 (C-14), 41.5 (C-18), 39.6 (C-8), 39.2 (C-4), 38.6 (C-1), 37.0 (C-10), 33.7 (C-21), 32.8 (C-29), 32.6 (C-7), 32.3 (C-22), 30.4 (C-20), 28.8 (C-23), 27.7 (C-15), 26.7 (C-2), 25.2 (C-27), 23.3 (C-30), 22.8 (C-16), 22.7 (C-11), 18.3 (C-6), 16.5 (C-26), 15.1 (C-25), 14.7 (C-24).

SS-5 (Betulinic acid)

White amorphous powder; yield: 28 mg; mp. 280-284 ^{o}C; $[\alpha]_D^{25}$: +10.1°(c 2.0, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3430 (OH), 1692 (C=O), 1645 (C=C), 707; HR-EIMS: 456.3588 (calculated for $C_{30}H_{48}O_3$, 456.3603). ^{1}H NMR (Py-d_5, 500 MHz) δ: 4.95 (1H, s, H-29α), 4.77 (1H, s, H-29β), 3.52 (1H, dt, J = 11.1, 3 Hz, H-19), 3.45 (1H, t, J = 7.2 Hz, H-3), 2.73 (1H, m, H-13), 2.63 (1H, m, H-16β), 2.25 (1H, m, H-22β), 2.24 (1H, m, H-21β), 1.94 (1H, m, H-12β), 1.88 (1H, m, H-18), 1.86 (2H, m, H-2), 1.79 (3H, s, H-30), 1.77 (1H, t, J = 11.5 Hz, H-3), 1.67 (1H, d, J = *12.8* Hz, H-1β), 1.57 (1H, m, H-22α), 1.56 (1H, m, H-6α), 1.55 (1H, m, H-16α), 1.53 (1H, m, H-21α), 1.45 (1H, m, H-7α), 1.43 (1H, m, H-11α), 1.38 (1H, m, H-6β), 1.38 (1H, m, H-9), 1.37 (1H, m, H-7β), 1.43 (1H, m, H-11α), 1.26 (1H, m, H-15α), 1.21 (3H, s, H-23), 1.21 (1H, m, H-12β), 1.07 (3H, s, H-27), 1.06 (3H, s, H-26), 1.01 (3H, s, H-24), 0.99 (1H, m, H-1α), 0.83 (3H, s, H-25), 0.82 (1H, m, H-5). ^{13}C NMR (Py-d_5, 125 MHz) δ: 178.84 (C-28), 151.32 (C-20), 109.93 (C-29), 78.14 (C-3), 56.64 (C-17), 55.94 (C-5), 50.98 (C-9), 49.80 (C-18), 47.78 (C-19), 42.88 (C-14), 41.14 (C-8), 39.52 (C-4), 39.30 (C-1), 38.64 (C-13), 37.54 (C-10), 37.56 (C-22), 34.86 (C-7), 32.89 (C-

16), 31.24 (C-21), 30.28 (C-15), 28.66(C-23), 28.31 (C-2), 26.14 (C-12), 21.22 (C-11), 19.50 (C-30), 18.81 (C-6), 16.43 (C-25), 16.43 (C-26), 16.33 (C-24), 14.92 (C-27).

SS-6 (Sclareol)

White crystalline solid; yield: 3.2 g; mp. 99-100 ^{o}C; $[\alpha]_D^{25}$:-2.9° (c 2.0, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3280, 2910, 1635, 1450, 1380, 1360, 910, 890, 708; HR-EIMS: 308.2688 (calculated for $C_{20}H_{36}O_2$, 308.2715). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.94 (lH, dd, J_{cis} = 10.8 Hz, J_{trans} = 17.4 Hz, H-14), 5.22 (lH, dd, J_{trans} = 17.4 Hz, J_{gem} = 1.6 Hz, H_{trans}-15), 4.99 (lH, dd, J_{cis} = 10.8 Hz, J_{gem} = 1.6 Hz, H_{cis}-15), 1.84 (1H, dt, *J* = 3.2, 12.2 Hz, H-7*e*), 1.64 (2H, H-12), 1.61 (1H, m, H-l*e*), 1.59 (2H, m, H-6*a*, *H*-2*a*), 1.46 (1H, m, H-11*a*), 1.42 (1H, m, H-7*a*), 1.40 (2H, m, H-2*e*, H-3*e*), 1.30 (2H, m, H-6*e*, H-11*e*), 1.25 (3H, s, Me-16), 1.15 (3H, s, Me-17), 1.14 (1H, m, H-9), 1.12 (1H, m, H-3*a*), 0.99 (1H, m, H-1a), 0.94 (1H, dd, *J* = 2.2, 12.0 Hz, H-5), 0.87 (3H, s, Me-19), 0.79 (6H, s, Me-18, Me-20). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 147.6 (C-14), 110.8 (C-15), 73.8 (C-8), 73.3 (C-13), 62.4 (C-9), 56.2 (C-5), 45.1 (C-12), 44.2 (C-7), 42.4 (C-3), 40.5 (C-1), 39.8 (C-10), 33.7 (C-4), 33.7 (C-19), 27.3 (C-16), 24.6 (C-17), 21.2 (C-6), 21.8 (C-18), 20.2 (C-11), 19.1 (C-2), 15.8 (C-20).

SS-7 (Salvigenin)

Yellow crystalline solid; yield: 67 mg; mp. 189-192 ^{o}C; IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 1665 (C=O), 1600, 1498, 1365, 830; UV (MeOH) λ_{max}: 330 and 277 nm; NaOMe: 375, 332 (sh), 295; $AlCl_3$: 360, 302 (sh), 235; $AlCl_3$/HCl: 354 (sh), 301, 262, 235; NaOAc: 376, 329, 277; NaOAc/H_3BO_3: 329, 276; HR-EIMS: 328.1264 (calculated for $C_{18}H_{16}O_6$, 328.0947); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.82 (2H, *d*, *J* = 8.8 Hz, H-2', H-6'), 7.00 (2H, *d*, *J* = 8.8 Hz, H-3', H-5'), 6.58 (1H, *s*, H-8), 6.53 (1H, *s*, H-3), 3.95 (3H, *s*, 4'-OMe), 3.91 (3H, *s*, 6-OMe), 3.88 (3H, *s*, 7-OMe); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 182.58 (C-4), 63.91 (C-2), 158.55 (C-5), 154.65 (C-4'), 153.13 (C-7), 151.98 (C-9), 132.54 (C-6), 124.9 (C-1'), 120. 62 (C-2', 6'), 112.44 (C-3', 5'), 105.4 (C-10), 103.3 (C-3), 90.5 (C-8), 60.77(C-4' OMe), 56.24 (C-6 OMe), 55.47 (C-7 OMe).

SS-8 (Acacetin)

Yellow crystalline solid; yield: 16 mg; mp. 167-172 ^{o}C; IR (KBr) ν_{max} cm^{-1}: 3325 (OH), 1675 (C=O), 1620, 1510, 1385, 832; UV (MeOH) λ_{max}: 329 and 269 nm; NaOMe: 341, 289; $AlCl_3$: 381, 278; $AlCl_3$/HCl: 352 (sh), 278; NaOAc: 330, 269; NaOAc/H_3BO_3: 333, 269; HR-EIMS: 298.0838 (calculated for $C_{17}H_{14}O_5$, 298.0841). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.86 (2H, dd, *J* = 1.96, 6.85 Hz, H-2', H-6'), 7.04 (2H, dd, *J* = 1.96, 6.85 Hz, H-3', H-5'), 6.57 (1H, s, H-3), 6.48 (1H, d, *J* = 2.45 Hz, H-8), 6.37 (1H, d, *J* = 2.45 Hz, H-6), 3.89 (3H, s, C-4' OMe), 3.87 (3H, s, C-7 OMe); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 182.40 (C-4), 164.30 (C-2), 156.40 (C-9), 154.60 (C-4'), 154.02 (C-5), 148.14 (C-7), 124.02 (C-1'), 120.33 (C-2', C-6'), 111.54 (C-3', C-5'), 103.40 (C-10), 103.33 (C-3), 98.05 (C-6), 93.50 (C-8), 61.50 (OMe), 56.15 (OMe).

SS-9 (Norartocarpetin)

Yellow Powder; yield: 10 mg; mp. 233-237 ^{o}C; IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1665 (C=O), 1622, 1515, 1392, 835; UV (MeOH) λ_{max}: 352, 270 and 251 nm; HR-EIMS: 286.0898 (calculated for $C_{15}H_{10}O_6$, 286.0477); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.75 (1H, d, *J* = 8.8 Hz, H-6′), 6.98 (1H, s, H- 3), 6.50 (1H, d, *J* = 2.4 Hz, H-3′), 6.44 (1H, dd, *J* = 2.4, 8.8 Hz, H-5′), 6.43 (1H, d, *J* = 2.2 Hz, H-8), 6.17 (1H, d, *J* = 2.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 181.8 (C-4), 164.3 (C-7), 161.9 (C-4′), 161.9 (C-2), 161.5 (C-5), 159.1 (C-9), 157.4 (C-2′), 129.7 (C-6′), 108.4 (C-1′), 108.1 (C-3), 106.8 (C-5′), 103.7 (C-10), 103.1 (C-3′), 98.7 (C-6), 93.7 (C-8).

Results and Discussion

Floral parts of *S. sclarea* were collected from IIIM Gene Bank, Kashmir, India in September 2012. The air dried powdered material was extracted with hexane and acetone. The concentrated hexane and acetone extracts were subjected to column chromatography over silica gel. Repeated column chromatography of hexane and acetone extracts using varied solvent polarity (hexane: ethyl acetate) and recrystallization techniques afforded nine compounds (Figure 1).

SS-1 was isolated as a colourless crystalline solid. The HR-EIMS showed a molecular ion peak at *m/z* 414.3837. From elemental analysis, HR-EIMS and other spectral data (^{1}H NMR, ^{13}C NMR), **SS-1** was assigned the molecular formula $C_{29}H_{50}O$. The IR spectrum showed a hydroxyl band at 3440 cm^{-1} and bands at 3060, 1655 and 815 cm^{-}1 due to trisubstituted double bond. **SS-1**, in its ^{1}H NMR, displayed resonance signals due to two quaternary methyl groups at δ 0.69 (3H, s, H-18) and 0.97 (3H, s, H-19) and three secondary methyl groups at δ 0.90 (3H, d, *J* = 6.5 Hz, H-21) and 0.87 (6H, d, *J* = 5.0, H-26, H-27), besides a resonance signal due to a primary methyl group at δ 0.80 (3H, *t*, H-29). The ^{1}HNMR spectrum of this compound displayed one resonance signal at δ 5.34 (1H, d, *J* = 3.1 Hz, H-6), due to olefinic proton. The ^{1}H NMR spectrum of this compound also showed one resonance corresponding to the proton connected to the C-3 (-OH carbon) which appeared as a triplet of doublet of doublets at δ 3.42. ^{13}C NMR-DEPT spectrum exhibited the presence of twenty nine carbon signal including six methyls, eleven

methylenes, ten methine and three quaternary carbons. This compound showed positive Liebermann-Burchard and Salkowski test specific for Δ^5 sterols [33, 34]. The MS spectrum showed characteristic fragment ions at *m/z* 399, 396, 381, 329 and 303. Fragment ions 329 and 303 are considered to be diagnostic for sterols having Δ^5-unsaturation [33, 34]. Comparison of physical characteristics and spectral data of CCE-1, with that reported in literature [35], confirmed it to be β-Sitosterol.

SS-2 was obtained as white crystalline powder with melting point 179-183 ^oC. The HREIMS showed a molecular ion peak at *m/z* 426.3848. From elemental analysis, HREIMS and other spectral data (^1H NMR, ^{13}C NMR), **SS-2** was assigned the molecular formula $C_{30}H_{50}O$. The compound responded positively to Liebermann-Burchard test indicating the presence of triterpene skeleton. The IR spectrum showed a hydroxyl band at 3320 cm^{-1} and the presence of gem dimethyl group (1380 and 1372 cm^{-1}). In the ^1H NMR spectrum, a multiplet centered at δ 5.53 integrating for one proton is assigned to an olefinic proton. Examination of the ^{13}C NMR spectrum also reveals the presence of two olefinic carbons at δ 124.5 and 139.6 ppm, which suggested an urs-12-ene triterpene skeleton. The other downfield signal at δ 3.85 (1H, J = 12 Hz) was assigned to proton which is which is attached to a hydroxylated carbon. ^{13}C NMR spectra revealed the presence of 30 carbon atoms including eight methyls, nine methylenes, seven methines and six quartenary carbons. The carbon bearing the hydroxyl group was located at δ 78.9 in ^{13}C NMR spectrum. Since this compound was earlier reported from the root parts of this plant species [23], it was identified as α-amyrin **f**rom the spectral evidence and comparing the physical data with the literature values [36].

SS-3 was obtained as colourless crystals with melting point 282-286 ^oC. The HREIMS showed a molecular ion peak at *m/z* 456.3594. From elemental analysis, HREIMS and other spectral data (^1H NMR, ^{13}C NMR), **SS-3** was assigned the molecular formula $C_{30}H_{48}O_3$. The compound was positive to Liebermann-Burchard test giving brownish violet colour indicating the presence of triterpene skeleton. The IR spectrum showed a hydroxyl band at 3440 cm^{-1}, an acid carbonyl band at 1700 cm^{-1} and bands at 1380 and 1372 cm^{-1} due to a gem dimethyl group. In the ^1H NMR spectrum, a multiplet centered at δ 5.50 integrating for one proton is assigned to an olefinic proton. Examination of the ^{13}C NMR spectrum also reveals the presence of two olefinic carbons at 125.7 and 139.3 ppm, which suggested an urs-12-ene triterpene skeleton. This was also supported by the presence of a 19β equatorial methyl group which is in close proximity to the double bond (γ and δ to C-13 and C-12 respectively) in urs-12-ens [37]. The other downfield signal at δ 3.46 (dd, J = 6.4, 9.6 Hz) was assigned to a proton connected to hydroxylated carbon (δ_C 79.0). The above interpretations were substantiated by 2D NMR experiments. The two methyl singlets at δ_H 1.23 and 1.01 (Me-23 and Me-24) showed strong correlations with the hydroxylated carbon resonating at δ_C 79.0 in its HMBC spectrum. Thus the signal at δ_C 79.0 was therefore assigned to C-3. Further ^{13}C NMR-DEPT spectra revealed the presence of 30 carbon atoms including seven methyls, one acid carbonyl, nine methylenes, seven methines and six quaternary carbons. In HMBC, the methyl protons resonating at δ_H 1.21 (Me-27) showed correlation with the olefinic carbon at δ_C 139.3 (C-13), the methyl protons at δ_H 1.04 and 1.21 (Me-26 and Me-27) showed correlation with a quaternary carbon resonating at δ_C 42.5 (C-14) and the methyl protons at δ_H 1.01 and 0.87 (Me-24 and Me-25) showed strong correlation with a carbon resonating at δ_C 52.8 (C-5). The presence of carboxylic group at C-17 was confirmed through HMBC interactions in which H-18 (δ 2.62) showed ^2J correlations with C-17 (δ 48.1) and ^3J correlation with C-28 (δ 179.8) of the carboxylic carbon atom. From the spectral evidence and comparing the physical data with the literature values [38], **SS-3** was identified as ursolic acid (Figure 2). This compound is reported for the first time from this plant species.

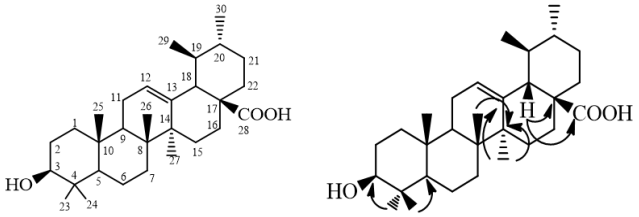

Figure 2. Structure of SS-3 (Ursolic acid) and its significant HMBC correlations.

SS-4 was obtained as colourless needles with melting point 307-311 ^oC. The HR-EIMS showed a molecular ion peak at *m/z* 456.3611. From elemental analysis, HR-EIMS and other spectral data (^1H NMR, ^{13}C NMR), **SS-4** was assigned the molecular formula $C_{30}H_{48}O_3$. The compound showed positive Liebermann-Burchard test indicating the presence of triterpene skeleton. The IR spectrum showed intense absorptions at 3450 cm^{-1} (OH group), 1700 cm^{-1} (carbonyl of carboxyl group) and 1660 and 820 cm^{-1} (trisubstituted double bond). The ^1H NMR spectrum revealed signals at δ 0.93, 0.97, 1.02, 1.02, 1.04, 1.24 and 1.30 which were assigned to protons of seven methyl groups. A doublet at δ 5.48 (1H, J = 3.4 Hz) was ascribed to olefinic proton while double doublet at δ 3.58 (1H, dd, J = 6.8, 9.4 Hz) was assigned to proton geminal to hydroxyl group. In the mass spectrum the prominent fragments at 248 ($C_{16}H_{24}O_2$) and 203 ($C_{15}H_{23}$) showed the characteristic

Δ^{12}-amyrin skeleton [36]. The ^{13}C NMR-DEPT spectrum of the compound indicated thirty carbon signals including six methyl, twelve methylene, six methine, and six quaternary carbon atoms. The chemical shifts at δ_C 180.4, 122.4 and 144.1 were the characteristic peaks for oleanolic type of skeleton, assigned to C-28, C-12 and C-13 respectively. The oxygen deshielded chemical shift at δ_C 78.5 was assigned to C-3. From the spectral evidence and comparing the physical data with the literature values [39], the **SS-4** was identified as oleanolic acid. This compound is reported for the first time from this plant species.

SS-5 was obtained as white amorphous powder with melting point 280-284 ^{o}C. The HR-EIMS showed a molecular ion peak at *m/z* 456.3588. From elemental analysis, HR-EIMS and other spectral data (^{1}H NMR, ^{13}C NMR), **SS-5** was assigned the molecular formula $C_{30}H_{48}O_3$. The compound was positive to Liebermann-Burchard test indicating the presence of triterpene skeleton. The IR spectrum showed a hydroxyl band at 3430 cm^{-1}. An absorption band at 1692 cm^{-1} was also observed indicating the presence of a carbonyl (C=O) group. The absorption band at 1645 was assigned to the double bond and a sharp band at 707 cm^{-1} is the characteristic absorption of =C-H bending. The ^{1}H NMR spectrum of **SS-5** displayed a pair of downfield signals centered at δ_H 4.95 (1H, s) and 4.77 (1H, s), attributable to an exo-methylene group which together with an allylic methyl singlet at δ_H 1.79 indicated an isopropenyl functionality. The presence of exo-methylene group was further supported by ^{13}C NMR-DEPT spectrum showing a downfield signal at δ_C 109.93 for an olefinic methylene group. A triplet at δ_H 3.45 (1H, t, J = 7.2 Hz) was assigned to proton geminal to hydroxyl group. In addition to this, the ^{1}H NMR spectrum of this compound exhibited signals for the protons of six methyl groups at δ_H 0.83, 1.01, 1.06, 1.07, 1.21 and 1.79. The ^{13}C NMR-DEPT spectra showed thirty carbon signals comprising of seven methyls, eleven methylenes, six methines and six quaternary carbons. The signal at δ_C 178.84 indicated the presence of carbonyl of carboxylic group. The signals at δ_C 151.32 and 109.93 were attributed for the presence of terminal olefinic carbons in the compound. These signals have been assigned to the isopropylene group at C-20 and C-29. The oxygen deshielded chemical shift at δ_C 78.14 was assigned to C-3. The ^{1}H and ^{13}C NMR data indicated the compound as a pentacyclic lupane triterpenoid and comparison of its physical and spectral data with published values confirmed the identity of **SS-5** as 3β-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) [40, 41]. This compound is reported for the first time from this plant species.

SS-6 was isolated as a colourless crystalline solid. The HR-EIMS showed a molecular ion peak at *m/z* 308.2688. From elemental analysis, HR-EIMS and other spectral data (^{1}H NMR, ^{13}C NMR), it was assigned the molecular formula $C_{20}H_{36}O_2$. The IR spectrum showed a hydroxyl band at 3280 cm^{-1} and bands at 1635 and 708 cm^{-1} due to double bond and =C-H bending respectively. In the ^{1}H NMR spectrum of **SS-6**, the signals at δ 5.94 (lH, dd, J = 10.8, 17.4 Hz), 5.22 (lH, dd, J = 17.4, 1.6 Hz) and 4.99 (lH, dd, J = 10.8, 1.6 Hz), were considered for the protons of a terminal olefinic moiety. The four signals at δ_H 1.25 (3H, s), 1.15 (3H, s), 0.87 (3H, s) and 0.79 (6H, s) were attributable to five methyl groups. The ^{13}C NMR-DEPT spectra showed twenty carbon signals comprising of five methyls, eight methylenes, three methines and four quaternary carbons. The signals at δ_C 147.6 and 110.8 indicated the presence of a terminal olefinic moiety in the molecule. These signals have been assigned to C-14 and C-15 respectively. The oxygen deshielded chemical shift at δ_C 73.8 and 73.3 were assigned to C-8 and C-13 respectively.

^{1}H and ^{13}C NMR gave evidence of a labdane-type diterpene containing the structural elements of an exo-methylene and two oxygen deshielded carbon atoms and comparison of its physical and spectral data with that reported in literature confirmed the identity of **SS-6** as (labd-14-ene-8,13-diol) (Sclareol) [42]. This compound was earlier reported from this plant species [23].

SS-7 was obtained as yellow crystalline solid with melting point 189-192 ^{o}C. The HR-EIMS showed a molecular ion peak at *m/z* 328.1264. From HR-EIMS and other spectral data (^{1}H NMR, ^{13}C NMR), **SS-7** was assigned the molecular formula $C_{18}H_{16}O_6$ and indicated eleven degrees of unsaturation. The compound responded positively to Shinoda test (orange colour), sulphuric acid test (yellow colour) and gave light blue colour in Gibbs test indicating the presence of flavonoid skeleton. IR spectrum exhibited a hydroxyl band at 3450 cm^{-1} and a carbonyl band at 1665 cm^{-1}. The UV spectrum of **SS-7** showed absorption maxima at 330 and 277 nm characteristic of flavonoids [43]. A bathochromic shift of 24 nm (354 nm) in band-I using $AlCl_3$/HCl indicated the presence of a free 5-hydroxyl group [44] and the absence of any shift in band-II using NaOAc indicated the absence of free 7-hydroxyl group [45]. The ^{1}H NMR spectrum showed signals for three methoxyls at δ_H 3.95, 3.91, 3.88 assigned to 4'- OMe, 6-OMe and 7-OMe respectively. A pair of doublets (J = 8.8 Hz each) observed at δ_H 7.82 and 7.00 integrating for four protons were assigned to H-2', H-6' and H-3', H-5' respectively. The two other singlets at δ_H 6.58 and 6.53

were attributed to H-8 and H-3 respectively. The ^{13}C NMR-DEPT spectra showed the presence of eighteen carbons comprising of six methines, nine quaternary and three methoxyl carbons. The signal at δ_C 182.58 was assigned to carbonyl carbon (C-4) and the signals at 163.91, 158.55, 154.65, 153.13 and 151.98 were assigned to oxygen deshielded carbons attributed to C-2, C-5, C-4', C-7 and C-9 respectively. From the spectral evidence and comparing the physical data with the literature values [46], **SS-7** was identified as 5-hydroxy-4', 6, 7-trimethoxy flavone (Salvigenin). This compound is reported for the first time from this plant species.

SS-8 was isolated as yellow crystalline needles with melting point 167-172 ^oC. The HR-EIMS showed a molecular ion peak at *m/z* 298.0838. The molecular formula $C_{17}H_{14}O_5$ was established from HR-EIMS, elemental analysis and other spectral data (^1H NMR, ^{13}C NMR). This compound responded positively to Shinoda test (orange colour), sulphuric acid test (yellow colour) and Gibbs test indicating the presence of flavonoid skeleton. IR spectrum exhibited a prominent hydroxyl band at 3225 cm^{-1} and a carbonyl band at 1675 cm^{-1}. The UV spectrum showed absorption maxima at 329 and 269 nm which is the characteristic feature of flavonoids [43]. A bathochromic shift of 23 nm (352 nm) in band-I using $AlCl_3$/HCl (shift reagent) indicated the presence of a free 5-hydroxyl group [44] and the absence of any shift in band-II using NaOAc indicated the absence of free 7-hydroxyl group [45]. The ^1H NMR spectrum showed two singlets at δ_H 3.89 and 3.87 integrating for three protons each assigned to C-4' and C-7 methoxyls respectively. A singlet at δ_H 6.57 integrating for one proton can be attributed to the flavone proton (H-3). A pair doublets at δ_H 6.48 (J = 2.45 Hz) and 6.37 (J = 2.45 Hz) each integrating for one proton were assigned to H-8 and H-6 protons respectively. The ^{1}HNMR spectrum also exhibited a pair of double doublets at δ_H 7.86 (J = 1.96, 6.85 Hz) and 7.04 (J = 1.96, 6.85 Hz) each integrating for two protons, assigned to H-2', H-6' and H-3', H-5' protons respectively. The ^{13}C NMR-DEPT spectra showed the presence of seventeen carbons comprising of seven methines, eight quaternary and two methoxyl carbons. The signal at δ_C 182.40 was assigned to carbonyl carbon (C-4) and the signals at δ_C 164.30, 156.40, 154.60, 154.02 and 148.14 were assigned to oxygen deshielded carbons attributed to C-2, C-9, C-4', C-5 and C-7 respectively. The two up-field signals at δ_C 61.50 and 56.15 were assigned to two methoxyl carbons. From the spectral evidence and comparison of physical data with that of literature [47], SS-8 was identified as 5-hydroxy-7, 4'-dimethoxy flavone (Acacetin). This compound is reported for the first time from this plant species.

SS-9 was obtained as a yellow amorphous powder with melting point 233-237 ^oC. It exhibited a molecular ion peak at m/z 286.0898 in HREIMS, consistent with molecular formula of $C_{15}H_{10}O_6$. This compound showed positive Shinoda test (orange colour), sulphuric acid test (yellow colour) and Gibbs test indicating the presence of flavonoid skeleton. IR spectrum exhibited a prominent hydroxyl band at 3400 cm^{-1} and a chelated carbonyl band at 1665 cm^{-1}. The UV spectrum exhibited maxima at 251, 270, and 352 nm characteristic of flavonoids [43]. The ^1H NMR spectrum of **SS-9** showed signals for six aromatic protons. The ^1H NMR spectrum revealed the presence of ABX type protons at δ_H 7.75 (1H, d, J = 8.8 Hz), 6.50 (1H, d, J = 2.4 Hz) and 6.44 (1H, dd, J = 2.4, 8.8 Hz) assignable to H-6′, H-3′ and H-5′ protons of 2′, 4′-dihydroxy-substituted B ring of the flavone nucleus. A pair doublets for two weakly meta-coupled protons at δ_H 6.43 (J = 2.2 Hz) and 6.17 (J = 2.2 Hz) each integrating for one proton were assigned to H-8 and H-6 protons respectively. In addition to this, a singlet at δ_H 6.98 integrating for one proton can be attributed to the flavone proton (H-3). The ^1H and ^{13}C NMR data including DEPT experiments showed the presence of fifteen carbon atoms including six methines and nine quaternary carbons. The signal at δ_C 181.80 was assigned to the chelated carbonyl carbon (C-4) and the signals at δ_C 164.30, 161.9, 161.9, 161.5, 159.1 and 157.4 were assigned to oxygen deshielded carbons attributed to C-7, C-4′, C-2, C-5, C-9 and C-2′ respectively. From the spectral evidence and comparison of physical data with that of literature [48], SS-9 was identified as 5, 7, 2', 4'-tetrahydroxy flavone (Norartocarpetin). This compound is reported for the first time from this plant species.

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