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Highlights

- Eight compounds were isolated from *Fagonia scabra* for the first time
- Six compounds were isolated from genus *Fagonia* for the first time
- Luteolin-7-*O*-*β*-D-glucopyranoside was a chemotaxonomic marker of *F. scabra*
- It is the first luteolin flavonoid isolated from Zygophyllaceae family
- Saponins from *F. scabra* were compared with other saponins from *Fagonia* species.

Abstract

This article reports on the structure of triterpenoids saponins (**1-49**) isolated from *Fagonia* species. In addition, it describes one known flavonoid (**50**) with luteolin as aglycone and seven known triterpenoid saponins (**26, 51-56**) isolated from the aerial parts of *Fagonia scabra* Forssk. Compounds **50-56**, were isolated for the first time from the genus *Fagonia*, while compounds **50-53**, **55-56** have never been encountered in the Zygophyllaceae family. The chemotaxonomic relationship between *F. scabra* and other *Fagonia* species was also discussed.

Keywords : *Fagonia scabra*, *Fagonia microphylla*, Zygophyllaceae, Triterpenoid saponins, luteolin

1. Subject and source

The Zygophyllaceae family is composed of approximately 27 genera and 285 species, mostly distributed in hyperarid and arid zones of the old and new world (Engler, 1931; Sheahan and Chase, 1996; Beier, 2003). Among these, *Fagonia* is one of the main genera with 34 desert plant species (Beier, 2003; Beier et al., 2004), and is represented by 17 species in the flora of Algeria (Quezel and Santa, 1963). Several of these are used in folk medicine, for their anti-inflammatory activity, analgesic and anti-pyretic effects, thrombolytic effects and antioxidant activity (Chopra et al., 1982; Saeed Asif and Sabir Wahid, 2003; Kasture et al., 2014). They are also used as popular remedies for the treatment of various skin lesions, and digestive disorders (Chopra et al., 1982).

Aerial parts of *Fagonia scabra* Forssk (= *F. microphylla* Pomel as synonym) were collected near Ghardaia (Algerian Septentrional Sahara) in April 2009. The botanical identification was made by Pr. Gerard De Bélair (University of Annaba, Algeria). A voucher specimen (ZKFm04/09) was deposited in the Herbarium of the Biology Department (Université Mentouri-Constantine, Algeria).

2. Previous work

Previous phytochemical studies on *Fagonia* species demonstrated the presence of flavonoids (Saleh et al., 1990; Al-Wakeel 1992; El-Wakil and Eman, 2007), diterpenoids (Abdel-Kader et al., 1993; Perrone et al. 2007; Sallam et al., 2014), sterols glycosides (Shoeb et al. 1994), triterpenes (Anjum et al., 2007; Saleem et al., 2014; Farheen et al., 2015) and saponins (Ansari et al., 1988; Miyase et al., 1996a; Melek et al., 2000; Batterjee et al., 2002; Sallam et al., 2014). A total of forty-nine triterpenoid saponins (**1-49**) have been identified in the five investigated *Fagonia* species, *F. arabica* L. (Miyase et al., 1996a; Shoeb et al, 1994; Perrone et al., 2007), *F. cretica* L. (Abdel Khalik et al., 2000a,b; Batterjee et al., 2002; Anjum et al., 2014; Saleem et al., 2014), *F. glutinosa* Delile (Melek et al., 2000), *F. indica* Burm.f. (Ansari et al., 1987,

1988; Shaker et al., 1999, 2000, 2013; Farheen et al., 2015), and *F. mollis* Delile (Sallam et al., 2014). Their structures are reported in Table 1 and Fig. 1.

3. Present study

Powdered air–dried aerial parts of *F. scabra* (1 kg) were extracted by petroleum ether (15 L). The defatted powder was then macerated in MeOH-H_2O (8:2, 12 L) for 24 h and refluxed for 3 h to afford 89.3 g of a dark residue after filtration and solvent evaporation. The residue was dissolved in MeOH (600 ml) and precipitated by addition of a large excess of Me_2CO (3 L). The resulting precipitate was filtered off, dried over KOH and dissolved in H_2O and dialyzed against pure distilled H_2O in seamless cellulose tubing (Spectra/por dialysis membrane, MCWO 6-8.0000, Spectum lab, France) under stirring during 48 h. The content of the tubes was freeze-dried to afford 3.6 g of a saponin mixture. This crude mixture was subjected to *vacuum* liquid chromatography over RP-18 using a gradient of MeOH-H_2O (4:6, 6:4, 8:2 and 0:1, each 250 ml) to give four fractions [F_1: 0.93 g, F_2: 1.19 g, F_3: 1.3 g, F_4: 0.16 g]. Fraction F_3 (1.3 g) was purified by silica gel CC, eluted with a $CHCl_3$-MeOH-H_2O gradient mixture [95:5:0-70:30:5], to afford pure compound **50** (600 mg) as a yellow precipitate. Fractions [36-46] (451 mg), eluted with $CHCl_3$-MeOH (9:1), were purified by semi prep HPLC using an isocratic program of 44% MeCN in H_2O during 10 min to yield **51** (10 mg). Fractions [47-89] (334 mg), eluted with $CHCl_3$-MeOH (8:2), were purified by semi prep HPLC using an isocratic eluent of 36% MeCN in H_2O during 20 min to give **52** (17.2 mg), **53** (9.6 mg), **54** (50 mg), and **51** (15 mg). Saponin **55** (15 mg) was purified from fractions [90-120], eluted with $CHCl_3$-MeOH (7:3), by semi-prep HPLC using a linear gradient from 36% to 42 % MeCN in H_2O during 15 min. Fraction F_4 (0.16 g) was subjected to silica gel CC using a gradient of $CHCl_3$-MeOH-H_2O [9:1:0 to 12:8:1]. Fractions [39-49] (17 mg) eluted with $CHCl_3$-MeOH (85:15) were purified by RP-18 CC, using a gradient of MeOH-H_2O [4:6 to 6:4] to give **26** (6 mg) and **56** (2.2 mg).

The structures of the isolated compounds were determined by 1D and 2D NMR experiments, and by comparison with data from the literature. Compound **50** was identified as luteolin-7-*O*-*β*-D-glucopyranoside (Agrawal, 1989), and was the major compound of the hydromethanolic extract. Other compounds were chikusetsaponin IVa (**51**) (Lin et al., 1976), spinasaponin A 28-*O*-*β*-D-glucopyranoside (**52**) (Paphassarang et al., 1989), pseudoginsenoside RT1 (**53**) (Tanaka et al., 1985), quinosid D (**54**) (Mizui et al., 1990), 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranosyl-oleanolic acid (**55**) (Ridout et al., 1994), matesaponin 1 (**26**) (Miyase et al., 1996b), and matesaponin B (**56**) (Sachiko et al., 2009) (Fig. 2)**.**

4. Chemotaxonomic significance

The present study reports the isolation and structural elucidation of one flavonoid (**50**) and of seven triterpenoid saponins (**26, 51-56**) from the hydromethanolic extract of the defatted aerial parts of *F. scabra* (Fig. 2)**.** Compounds **50-56**, were isolated for the first time from the genus *Fagonia*. Compounds **50-53** and **55** have not been reported from any species in Zygophyllaceae family.

Triterpenoid saponins are bitter compounds frequently present in plants from arid countries to deter predation. They are representative secondary metabolites of *Fagonia* species and 49 of them (Fig 1) have been identified from five *Fagonia* species (**Table 1)**. These saponins were mono-or disaccharides of oleanane triterpenoid, such as oleanolic acid (**1**, **13-19, 36, 41**), hederagenin (**2, 33-35**), and 27-hydroxyoleanolic acid (**3-4, 20-21, 37-38**), or ursane triterpenoid, such as ursolic acid (**24-30, 43-44**), 27-hydroxyursolic acid (**22-23, 31-32**), and quinovic acid (**6-7, 10-12, 42**). The carbonyl in C-28 position was free or esterified by a *β*-D-glucopyranosyl unit or by a gentiobiosyl unit (*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl) (Fig.1). The hydroxyl in C-3 position was also glycosylated by a *β*-D-glucopyranosyl unit (**2, 6-7, 10, 45-47**) or by an *α*-L-arabinopyranosyl which can be substituted in C-2 and/or C-3

positions by a *β*-D-glucopyranosyl and/or a *β*-D-xylopyranosyl units (**11-32, 35-40, 43-44**). Saponins isolated from *Fagonia cretica* (Batterjee et al., 2002) were slightly different with an *α*-L-rhamnopyranosyl substituted in C-4 position by a *β*-D-glucopyranosyl unit (**33**) or a disaccharide moiety (*β*-D-glucopyranosyl-(1→5)-*α*-L-arabinofuranosyl) (**34**). In fagonoside A (**49**) isolated from *F. cretica* (Anjum et al., 2014) a 6-deoxy-*α*-L-talopyranosyl was attached to the 3*β*,22*α*-dihydroxyurs-12-en-28-oic acid. Sulfated saponins were described in *F. arabica* (Perrone et al. 2007) (**3-4, 8-9, 22-23**), *F. indica* (Shaker et al., 1999) (**43-44**), and *F. mollis* with quinovic acid (**7**) (Sallam et al., 2014). On the other hand three taraxastane saponins were characterized in *F. indica* (**45-46**) (Ansari et al., 1987; Farheen et al., 2015) with fagonicin or 3*β*,20*S*-dihydroxytaraxastane-28-al and two nahagenin derivatives were isolated from *F. arabica* (**8**) (Perrone et al. 2007) and *F. indica* (**48**) (Ansari et al., 1987).

Our investigation of *F. scabra* adds to this list five oleanane (**51-55**), and two ursane (**26**, **56**). triterpenoid saponins. Matesaponin 1 (**26**) with ursolic acid as aglycon was previously isolated from *F. indica* (Shaker et al., 2013) and *Larrea tridentate* (Sesse. & Moc. Ex DC.) Coville (Zygophyllaceae) (Jitsuno and Mimaki, 2010). Compound **56** is a 23-hydroxyursolic acid glycoside isolated from *Ilex paraguariensis* St. Hilaire (Aquifoliaceae) (Sachiko et al., 2009) and was isolated for the first time from a *Fagonia* species. This saccharide moiety is also present in matesaponin 1 (**26**) with an *α*-L-arabinopyranosyl attached to the position C-3 of the aglycone, which is a characteristic of this genus *Fagonia*. Except for quinosid D (**54**) previously isolated from *Larrea tridentate* (Zygophyllaceae) (Jitsuno and Mimaki, 2010), all others saponins (**51-53** and **55**) were isolated for the first time in Zygophyllaceae. Compound **51** have been isolated from *Panax japonica (=P. japonicus* (T. Ness) C.A.Mey*)* (Araliaceae) (Lin et al., 1976) and *Beta vulgaris* L. (Chenopodiaceae) (Yoshikawa et al., 1996). Compounds **52** and **53** have been isolated from *Panax* species (Araliaceae) (Paphassarang et al., 1989), whereas compound **55** was isolated from *Beta vulgaris* (Chenopodiaceae) (Ridout et al., 1994) for

example. Compounds **51-55** were glycosides of oleanolic acid as in compounds **1**, **13-19, 36**, and **41**, but differ by their saccharide moieties. A *β*-D-glucuronopyranosyl unit, substituted in position C-2 and/or C-3 by *β*-D-glucopyranosyl and/or *β*-D-xylopyranosyl units, was attached to the hydroxyl in position C-3 of the aglycone (Fig.2). This significant chemical difference indicates a different chemotaxonomic feature and may contribute to enhancing our understanding of the taxonomy and evolution of the genus *Fagonia*.

In addition to saponins, the *Fagonia* species showed remarkably a homogeneous flavonoid profile in which quercetin, kaempferol, isorhamnetin, herbacetin, and herbacetin 8-methyl ether and their glycosides are predominant (Saleh et al., 1990; Abdel-Kader et al., 1993; El-Wakil and Eman, 2007). Compound **50**, luteolin-7-*O*-*β*-D-glucopyranoside, has been reported to occur in many plants (Agrawal, 1989). To our knowledge, this is the first flavonoid with luteolin as aglycone isolated from *Fagonia* genus and Zygophyllaceae family (Saleh et al., 1990; Al-Wakeel, 1992).

In summary, this study extends the knowledge of the metabolites of *F. scabra*, suggesting that they might be regarded as chemotaxonomic markers. *F. scabra* is endemic to the Saharo-Sind region and Western Moroccan as well as *F. glutinosa*, *F. arabica* and *F. cretica* but they are not in the same clade (Beier et al., 2004), which is confirmed by the presence of quite a different secondary metabolism.

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Table 1.Triterpenoid saponins of *Fagonia* species

Fagonia sp	Triterpenoid saponin	N°	Reference
F. arabica	3-*O*-β-D-glucopyranosyl-oleanolic acid	**1**	Shoeb et al. 1994
	3-*O*-α-L-arabinopyranosyl-quinovic acid-28-*O*-β-D-glucopyranoside	**11**	El-Wakil and Eman , 2007; Miyase et al., 1996a
	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-quinovic acid-28-*O*-β-D-glucopyranoside	**12**	
	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-oleanolic acid	**13**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-oleanolic acid	**15**	El-Wakil and Eman, 2007; Miyase et al., 1996a
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-oleanolic acid	**16**	Miyase et al., 1996a
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside	**17**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside	**18**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-27-hydroxyoleanolic acid-28-*O*-β-D-glucopyranoside	**21**	El-Wakil and Eman, 2007
	3-*O*-[2-*O*-sulfo-α-L-arabinopyranosyl]-27-hydroxyursolic acid	**22**	Perrone et al., 2007
	3-*O*-[2-*O*-sulfo-α-L-arabinopyranosyl]-27-hydroxyursolic acid-28-*O*-β-D-glucopyranoside	**23**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside	**28**	Miyase et al., 1996a
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-27-hydroxyursolic acid-28-*O*-β-D-glucopyranoside	**32**	
	3-*O*-sulfonyl-27-hydroxyoleanolic acid	**3**	Perrone et al., 2007
	3-*O*-sulfonyl-27-hydroxyoleanolic acid-28-*O*-β-D-glucopyranoside	**4**	
	3β,23-disulfonyl-nahagenin	**8**	
	3,23-disulfate ester of3β,23-dihydroxyolean-13(18)-en-28-oic acid-28-*O*-β-D-glucopyranoside	**9**	
F. cretica	3-*O*-β-D-glucopyranosyl-quinovicacid	**6**	Saleem et al., 2014
	3-*O*-β-D-glucopyranosyl-hederagenin	**2**	Batterjee et al., 2002
	3-*O*-β-D-glucopyranosyl-quinovic acid-28-*O*-β-D-glucopyranoside	**10**	Saleem et al., 2014
	3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-hederagenin	**33**	Batterjee et al., 2002
	3-*O*-β-D-glucopyranosyl-(1→5)-α-L-arabinofuranosyl-(1→4)-α-L-rhamnopyranosyl-hederagenin	**34**	
	3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranoside	**35**	Abdel Khalik et al., 2000a
	3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-oleanolic acid-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-] ester	**36**	
	3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-27-hydroxyoleanolic acid-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-]ester	**37**	
	3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-27-hydroxyoleanolic acid-28-*O*-[β-D-glucopyranosyl-(1→6) -β-D-glucopyranosyl-] ester	**38**	Abdel Khalik et al., 2000b
	3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-olean-12-en-27-al-28-oic acid-28- *O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-] ester	**39**	Abdel Khalik et al., 2000a
	3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl- olean-12-en-27-al-28-oic acid-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-] ester	**40**	Abdel Khalik et al., 2000b
	3β-*O*-[(6-deoxy-α-L-talopyranosyl)oxy]-22α-hydroxyurs-12-en-28-oic acid (fagonoside A)	**49**	Anjum et al., 2014
F. glutinosa	3-*O*-α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside	**24**	Melek et al., 2000
	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-ursolicacid	**25**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-ursolic acid	**27**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]-α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside	**29**	
	3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-27-hydroxyursolic acid-28-*O*-β-D-glucopyranoside	**31**	
	3-*O*-β-D-glucopyranosyl(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-27-hydroxyoleanolic acid-28-*O*-β-D-glucopyranoside	**20**	
F. indica	oleanolic acid-28-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-] ester	**41**	Shaker et al., 2000
	3-*O*-α-L-rhamnopyranosyl-quinovic acid-28- *O*-β-D-glucopyranoside	**42**	
	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside	**14**	Shaker et al., 2013
	3-*O*-β-D-glucopyranosyl-(1 →3)-α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside	**26**	
	3-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside (indicasaponin B)	**19**	
	3-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)-]α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside (indicasaponin A)	**30**	
	3-*O*-β-D-4-*O*-sulfonylglucopyranosyl-(1→3)-α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside (indicasaponin C)	**43**	Shaker et al., 1999
	3-*O*-β-D-4-*O*-sulfonylglucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)-]α-L-arabinopyranosyl-ursolic acid-28-*O*-β-D-glucopyranoside (indicasaponin D)	**44**	
	3-oxo-12-en-23-*O*-β-D-glucopyranosyl-27-hydroxyolean-28-oic acid (indicacin)	**5**	Farheen et al., 2015
	3β,*O*-β-D-glucopyranosyl-23-hydroxytaraxer-20-en-28-oic acid	**45**	Farheen et al., 2015
	23,28-di-*O*-β-D-glucopyranosyl-23-hydroxytaraxer-20-en-28-oic acid	**46**	Farheen et al., 2015; Ansari et al., 1987
	3β,28-di-*O*-β-D-glucopyranosyl-23-hydroxytaraxer-20-en-28-oic acid	**47**	Ansari et al., 1987
	21,22α-epoxy-23-*O*-β-D-glucopyranosyl-nahagenin	**48**	Ansari et al., 1988
F.mollis	3-*O*-β-D-glucopyranosylquinovicacid	**6**	Sallam et al., 2014

	3-*O*-β-D-2-*O*-sulfonylglucopyranosylquinovic acid	**7**

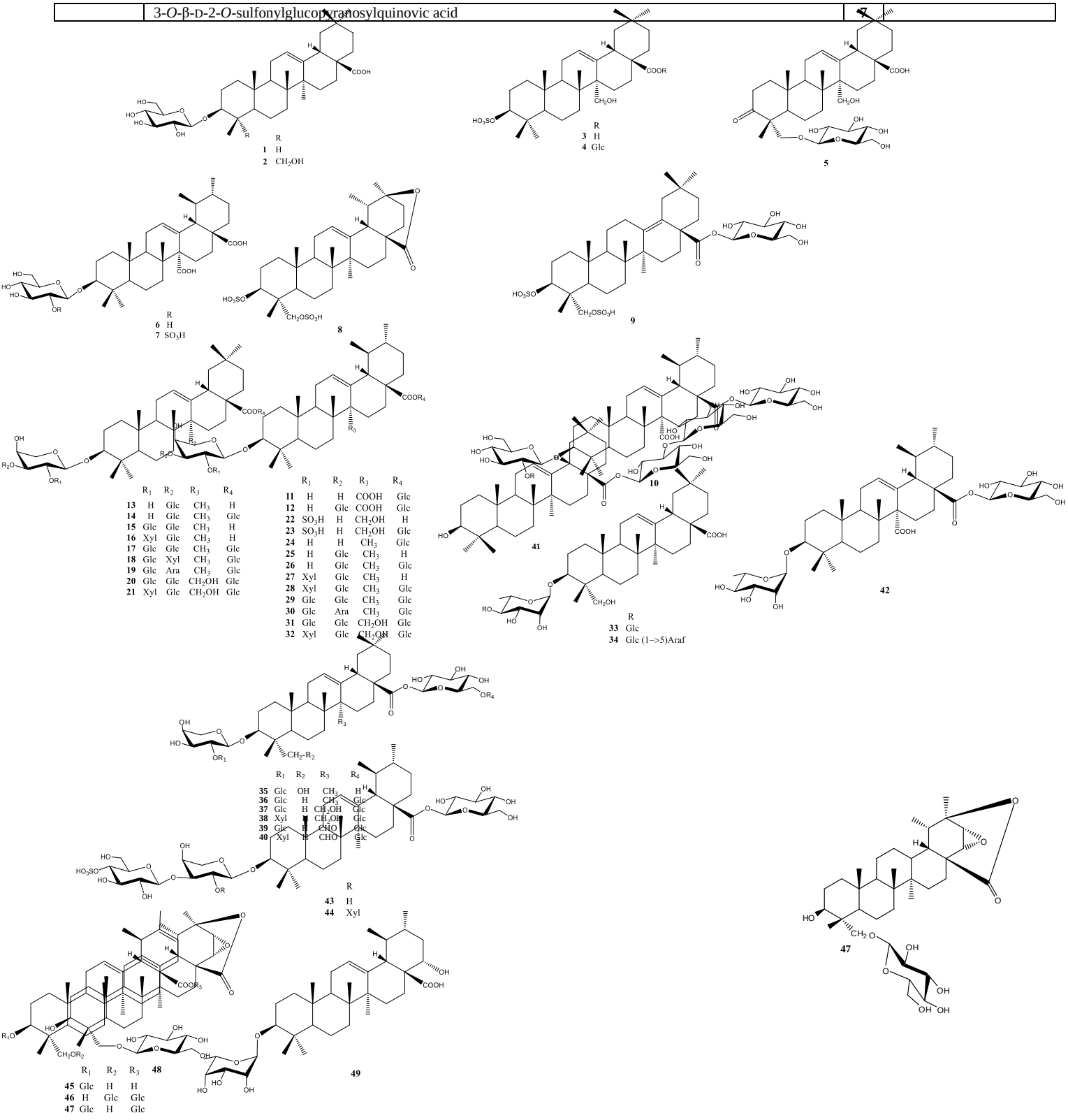

Fig.1: Structures of triterpenoid saponins isolated from *Fagonia* species

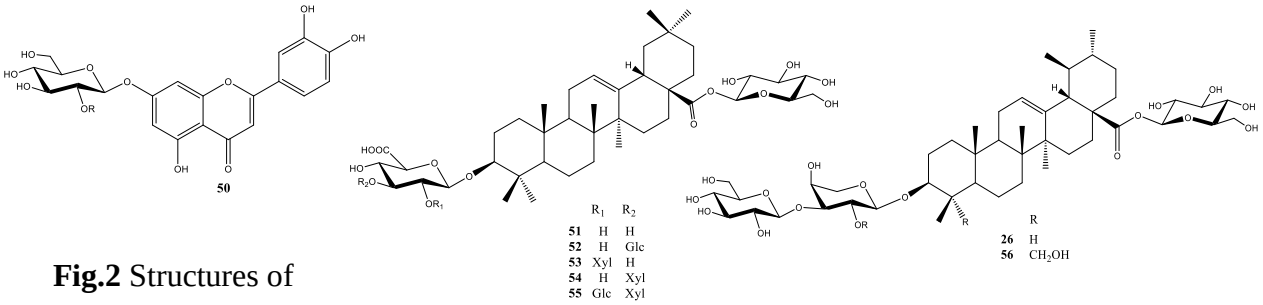

Fig.2 Structures of compounds (**26, 50–56**) isolated from *Fagonia scabra*