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Extraction, isolation and quantification of saponin from *Dodonaea viscosa* JACQ

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Abstract
The isolation of saponin from various solvent extract of the plant, *Dodonaea viscose* were studied. *Dodonaea viscosa* is a shrub belonging to the family *Sapindaceae*, and is commonly called as hop bush. Saponins are heterosides of plant origin and the type of molecules has an interesting pesticide potential. The saponin was isolated from the leaves, which is further purified with the help of solvent separation method and that shows the positive result for preliminary tests such as foam test, Lieberman's test. The isolated extract was purified by analytical method (Edeoga, *et al.*). The IR studies of saponin shows the peaks at-3069 (Aromatic –CH stretching), 2924 (-CH_3), 1636 (C=C Stretching), 1076 (C-O-C stretching of glycoside linkage of Oligosaccharide to sapogenin) which indicated the presence of functional group. The HPLC studies of saponin shows the peak at 205nm which get matched with the std. saponin and on the basis of that data reveals both qualitative and quantitative analysis of saponin. The amount of saponin present in *Dodonaea viscose* was found to be 173.25µg/mg.

Keywords: Saponin, *Dodonaea viscosa*, HPLC, IR

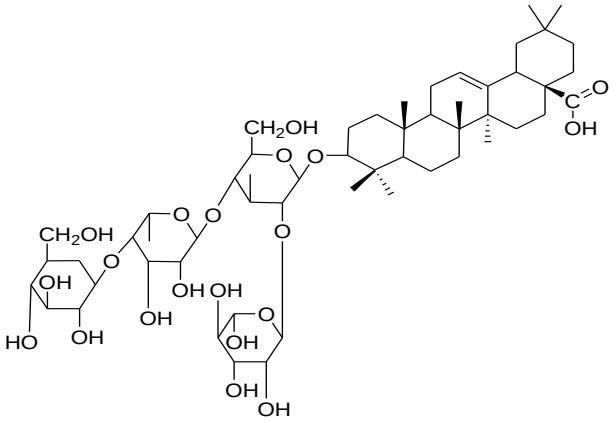
Introduction
Saponin has various types; it can be bond with glycosides that form soapy lathers when mixed and agitated with water, and have been used to treat diabetes; liver, hepatitis, cardiovascular as high blood pressure, high cholesterol and physical stress.
Among substances involved in plant defense, Saponins which are heterosides synthesized by several plants were reported to have a defensive role which was highlighted for the first time by (Appelbaum in 1969) [1]. *Saponins or saponosides* set up a large and frequent group of heterosides in plants and are characterized by their surface-active properties, Saponins dissolve in water by forming a foaming solution due to their tension-activity; hence, these substances take their name from Latin (*Sapo, saponis*: soap). Saponins are used for industrial as well as for pharmacological purposes. Several saponosides are used by pharmaceutical industry for obtaining drugs or by cosmetics industry for their detergent property (Sridhar *et al.,* 2012) [18].

Sources of saponin
(Dini, *et al.,* 2009) [7], reported in his study that saponins present widely in different plant families and also in food, such as peas, potatoes, sugar beets, asparagus, and beans differs in saponin contents in plant organs. Factors such as cultivar, age, geographical location and physiological state of plant determine the content of saponin. Total steroidal saponins are mainly distributed in Amaryllidaceae are, *Quillaja saponaria* and *Yucca schidigera*. Within plant families, saponins was found in various parts of plant, such as leaves, stems, fruits, bulbs, blossom and roots. (Dini *et al.,* 2001) [8] Observed the saponin content. (Man *et al.*, 2010) [13], isolated the saponin from different plant families such as *Agavaceae, Dioscoreaceae, Liliaceae, Solanaceae, Scrophulariaceae, Leguminose* and *Rhamnaceae*. Commercial saponins are mainly extracted from dessert plants, which revealed the presence of saponins in more than 100 families of plant, as evidenced by isolation of saponins from phytochemical studies of many plant species over the years.
Several saponosides substances are extracted from *Glycyrrhiza glabra, Agave attenuate, Panax ginseng, Saponaria officinalis* (Cheeke, 1971) [5]. *Lilium sativum* (De Geyter, 2007) [6] *Medicago sativa* (Pracros P., 1988) [15] and *Cestrum parqui* (Chaieb, 2009) [3]. In addition to their plant origin, saponin can be obtained from some marine animals. Commercial saponins are mainly extracted from dessert plants, which are *Quillaja saponaria* and *Yucca schidigera* (Cheeke, 2000) [4].

Steroid saponins are found in oats, capsicum pepper, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng.

Saponins are also found in defensive secretions of certain insects. Triterpenic saponin are isolated from Chrysomelidae especially the *Platyphora* genus. Species of this genus sequester, used saponins from their plant hosts to use them for their own defense (Plasman, *et al,* 2000) [14]. Bajad and Pardeshi [2], Studied the biopesticidal effect of natural saponin containing plant extract of *Acacia concinna* on pulse beetle, *Callosobruchus chinensis.* In the present study saponin were isolated from the extract of *Dodonaea viscosa* then purified and total contents of saponin were studied by analytical methods, IR and HPLC.

Structure of Saponin



Material and Methods

Extraction of Plant Material

The plant parts of *Dodonaea viscosa* were collected from Aurangabad and nearby areas. The plant material were cleaned, washed by tap water and dried in laboratory under shed at room temperature. The dried leaves were taken in the mixer cum grinder to make a fine powder and 20gms of powder was then extracted with ethanol, methanol and water solvent in a soxhlet apparatus for 24 hrs. The obtained extract from solvent after evaporation was used for investigative study. Saponin test used fresh and dried sample, chemicals test were carried out on the aqueous extract and on the powder sample using standard procedures to identify the saponin as described by (Sofofara 1993 and Harborne 1973) [17, 10].

Saponin Test with dry sample

0.5 gram of crude powder was shaken with water in a test tube and it was warmed in a water bath, the stable persistent froth, was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion, indicates the presence of saponin (Kapoor *et al.,* 1969; Smolenski *et al.,* 1974 and Edeoga *et al.,* 2005) [12, 16, 9].

Saponin test with fresh sample

10 gram of fresh sample (Plants), dissolve in 100ml distilled water (1:10), to blend, and filtered, the filtrate in the test tube, and it was warmed in water bath, the stable persistent froth, was mixed with 3 drop of olive oil and shaken vigorously, than observed for the formation of emulsion, indicate the presence of saponins (Edeoga *et al.,* 2005) [9] with some modified.

Terpenoid Test (Salwoski test)

5 ml of water extract from plant was mixed in 2 ml of chloroform, and concentrated 3 ml of H_2SO_4, and was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive result for the presence of terpenoid (Edeoga *et al.,* 2005) [9].

Isolation of saponin from plant extract

Determination of Saponin compound (Identification by analytical method). The samples were ground and 20 gram of each was put into conical flask and 100 ml of aqueous ethanol were added. The samples were heated over hot in water bath for 4 hours with continuous stirring at about 55ºC.Themixture was filtered and the residue re extract with another 200 ml 20% ethanol. the combined extract were reduced to 40 ml over water bath at about 90oC temperature was transferred into 250 ml separatory funnel and 20ml of diethyl ether was added shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.60 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponins content was calculated (Edeoga *et al.,* 2005) [9].

Observation and Results

Extracts obtained from soxhlet apparatus were purified and the amount of saponin was obtained. The qualitative preliminary test namely Froth test for saponin and Salwoski test for triterpene were taken, which indicates presence of saponin. IR spectra of saponin were taken to correlate the structure. FTIR spectra were recorded on a Perkin-Elmer FTIR spectrometer 65 in KBr pellets. A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle. Powder was compressed into a thin pellet using KBr press. Infrared spectra were recorded between 4000 − 500 cm $^{-1}$. The IR spectra of saponin shows the peaks at 3069 (Aromatic –CH stretching), 2924 (-CH_3), 1636 (C=C stretching), 1076 (C-O-C stretching of glycoside linkage of Oligosaccharide to sapogenin).

The HPLC studies of saponin shows the peak at 205 nm which gets matched with the standard saponin confirming the presence of saponin in isolated saponin samples of *Dodonaea viscosa.* The amount of saponin present in *Dodonaea viscosa* were calculated and % saponin was found to be 173.25µg/mg.

Table 1: The amount of saponin present in *Dodonaea viscosa* were calculated and % saponin was found to be 173.25µg/mg.

Plant	Solvent	Leaves extract in gms.	Saponin (%) By Analytical Method	Saponin (%) By HPLC
Dodonaea viscosa	Aqueous	2.940	1.7	Present
	Methanol	3.500	2.1	Present
	Ethanol	3.940	2.5	173.25µg/mg.

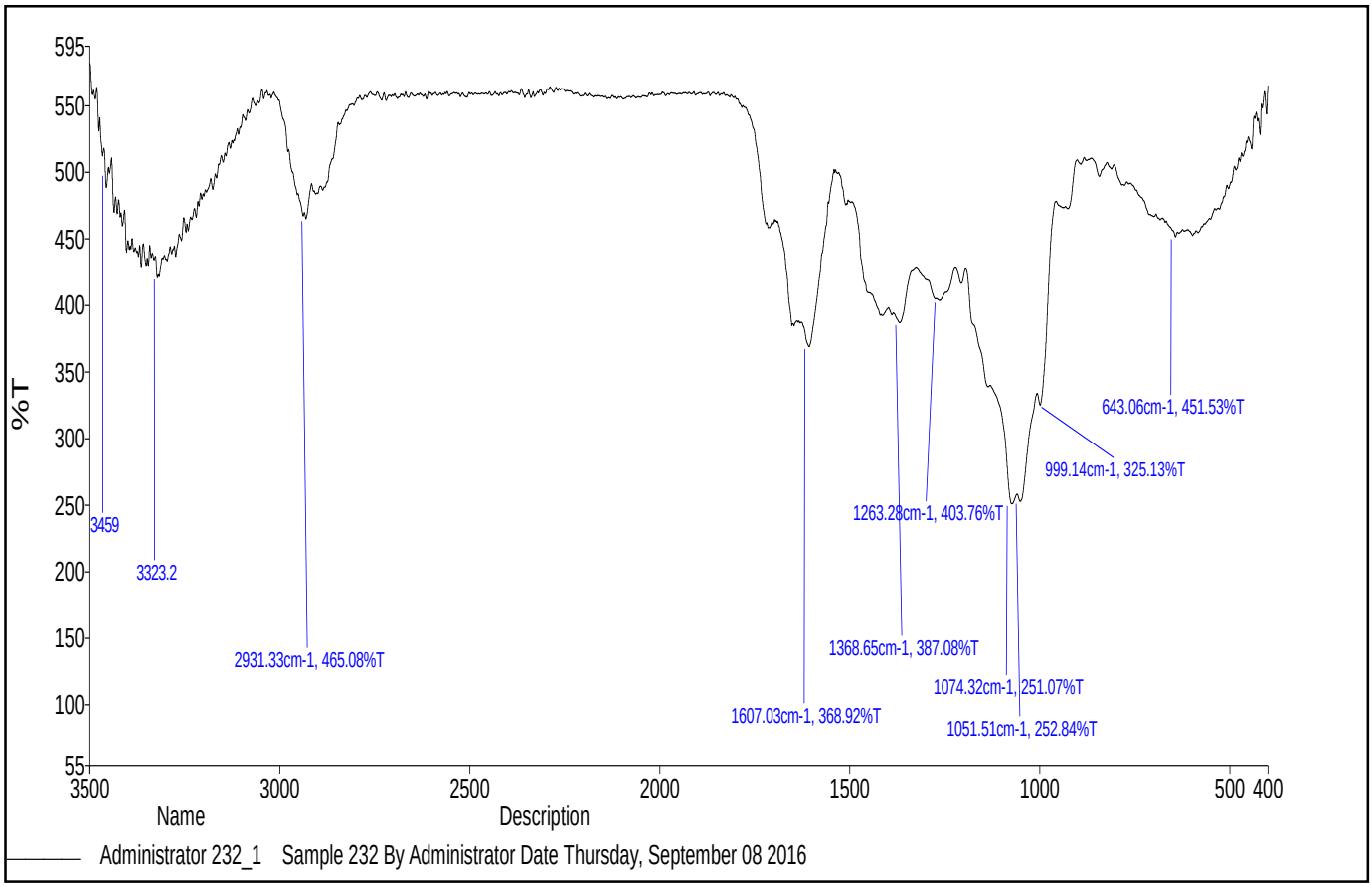


Fig 1: IR spectra of *Standard saponin*

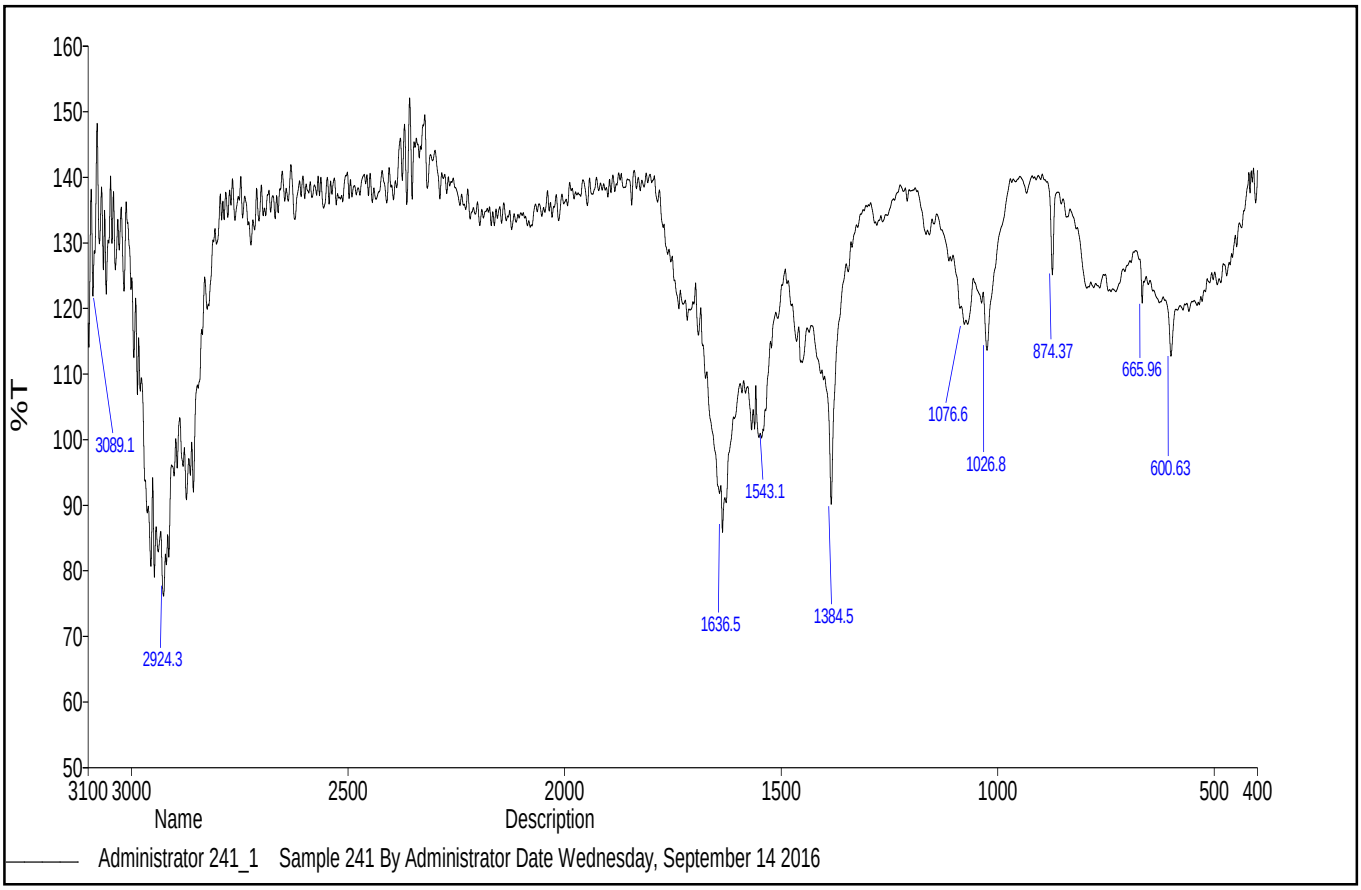


Fig 2: IR spectra of saponin extracted from *Dodonaea viscosa*.

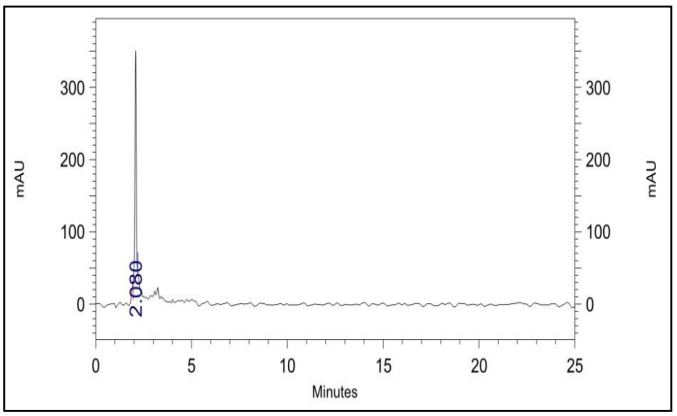


Fig 3: HPLC Chromatogram (monitored at 210 nm) of Standard Saponin

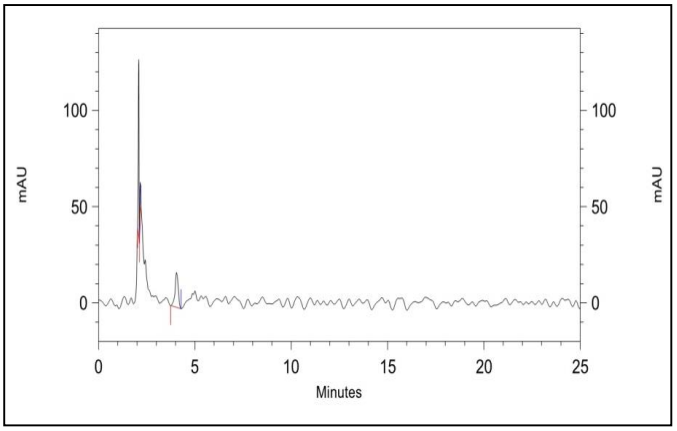


Fig 4: HPLC Chromatogram (monitored at 210 nm) of ethanol leaf extract of *Dodonaea viscosa* containing saponin

Discussion

Saponins are a class of secondary plant metabolites with diverse biological properties. They occur in a great number of plant species (mainly Angiosperms), both wild plants and cultivated crops. Triterpenoid saponins are mostly found in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocots (De Geyter, 2007) [6].

HPLC–DAD method is a powerful tool for content determination of saponins in camote tubers, due to its rapid analyses time, no sample consumption, good precision and accuracy, low cost of analyses and, last but not least, its widespread presence in analytical laboratories (Dini *et al.*, 2001) [8].The presence of terpenoids in *S. dulcis* has also been reported and this plant is widely used in herbal medicine (Hayashi *et al.*, 1993) [11]. Secondary substances in plants are known for a long time for their medicinal and pharmacological properties. These substances are necessary for the plant to evolve in a hostile environment. The plant can indeed use its secondary metabolites to be protected against several pest, animals and pathogenic microbose (Chaieb I., 2009) [3]. Saponins became an excellent model of study of natural substances with insecticidal effect due to their large spectrum of action and to the multitude of their physiological effects.

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