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A Study of Phytochemistry and Ethnobotanical Significance of *Adhatoda vasica* (L), Nees. (Syn- *Justicia adhatoda* L.) or Vasaka.

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Abstract-

Adhatoda vasica (L), Nees. (Syn- *Justicia adhatoda* L.) of family Acanthaceae is a shrub with opposite ascending branches and is considered the most significant medicinal plant, commonly known as Vasaka, Vasica, Adusa, and distributed in various regions of India as well as the World. The plant has been used in the indigenous system of medicine in India for nearly 2500 years. It is a well- known plant as a drug in Ayurvedic and Unani medicinal system. Traditionally it was used for the treatment of various acute and chronic diseases and showed strong pharmacological activity particularly for bronchial infections, cough, common cold, asthma, tuberculosis, reproductive disorders etc. All the parts of the plant are used by indigenous peoples or folklores to treat various diseases and many medicinal formulas have been developed by Ayurvedic experts due to its unique phyto chemical profile. In order to detect the phytochemistry various laboratory techniques can be used, one of them is a High performance liquid chromatography- Mass Spectrometry (HPLC-MS), generally known as LC-MS technique. LC–MS is a powerful analytical technique that combines the physical separation capabilities of HPLC with the mass analysis capabilities of MS. Coupled chromatography LC – MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. The leaf extract of vasaka was separated and charged ions detected by LC-MS that may be help to identify each separated component. The LC-MS data may be used to provide information about the molecular weight, structure, identity and quantity of specific sample components.

Keywords: Vasaka**,** Ayurveda, Bronchodilator, Alkaloids. Vasicine, Extract, HPLC-MS, Folklores, Chitrakoot.

Introduction-

Adhatoda vasica is commonly known as vasaka, adusa. malabar nut and it belongs to the family Acanthaceae. This is a vital plant, native of Asia that is widely spread in the area of the Indian subcontinent like Punjab, U.P, Bengal, Assam, Nepal and Sri Lanka. Along with that it covers Indian plains as well as ranges of Himalaya at an altitude of 1300 meter above sea level (Shoaib A.2021). It is also found in Malaysia, Myanmar, Singapore and many more countries around the globe (Singh et al., 2017). The plant is a small, evergreen, shrub with opposite branches, lance –shaped, exstipulate, dark green or yellowish green leaves, white flowers with an unpleasant smell and bitter taste. This plant mainly grows along the road or street as well as canals sides in all seasons of the year. It contains long opposite branches. The stem is woody from the abaxial side and herbaceous from the adaxial side. Flowers are large, dense borne in terminal spikes with large bracts, bisexual, zygomorphic, small, irregular, hypogynous, white, pink or purple in appearance with personate corolla. Leaves of the plant are simple, dark green or yellowish green, tapering base, reticulate, opposite, short peduncle, elliptic-lanceolate or ovate-lanceolate, hairy with breadth 4-7 cm and 7-19 cm long. The fruits of this plant are small, clavate and longitudinally capsulated having four globular seeds with length5-6 mm (Rao et al., 2013).

Fig.1 Twig of Adusa plant with inflorescence and white flowers with personate corolla

For several decades it was used as a medicine for the treatment of various ailments due to its versatile nature. This plant has been used for 2000 years to treat respiratory problems and it was also said by ancient Indians," No man suffering from phthisis needs despairs long as the Vasaka plant exists". The leaves of plant give a stimulant effect on the respiratory system. In Ayurveda medicinal system it is considered a prime plant to cure diseases like cough, symptoms of cold, asthma, bronchitis. This plant is treated like a mother to doctors in the

traditional medicinal system of India therefore known as Vaidyamata singhee in Sanskrit (Choudhary et al., 2021). The plant is known to have a rich source of alkaloids, phenolics flavonoids, carotene, essential oils, sterols etc. In Ayurveda medicinal system, it is used to cure various diseases like Shwasa (dyspnea), Kashaya (phthisis), Kasa (cough), raktapitta (hemorrhagic disease) Kamala (jaundice) and Kushtha (Padmaja et al., 2011). Richness in phytoconstituents makes adusa plant unique for various medicinal purposes. High performance liquid chromatography-Mass spectrometry (HPLC- MS) systems facilitate the analysis of samples that traditionally have been difficult to analyze. Despite the power and usefulness of gas chromatography-mass spectrometry (GC-MS), many compounds are impossible to analyze with GC-MS. LC-MS significantly expands the effective analytical use of mass spectrometry to a much larger number of organic compounds. LC-MS-based methods can be applied to most organic compounds. Sample types range from small pharmaceutical compounds to large proteins. Because it is a much more widely applicable method than GC-MS. LC-MS is suitable for the analysis of large, polar, ionic, thermally unstable and in volatile compounds. This study updates information on about phyto chemical profile of *A vasica* leaf extract through LC-MS. The study also focus on potential involvement of vasaka plant in traditional (Ethnobotanical) and medical therapy of various diseases.

Material and Methods-

1-Identification of plant

For identification of selected plant specimen first of all collect the plant sample, then make a herbarium sheet, and then send it to Botanical survey of India, central regional centre Prayagraj, India for the identification and authentication. The herbarium sheet is deposited in the herbaria of Botanical survey of India central regional centre Prayagraj, India for future reference.

2- Preparation of plant sample

In order to study phyto chemical profile of the plant, first of all we collected plant material (leaves) of selected plant *A vasica* in required amount. Then leaves were washed thoroughly in tap water to remove dust particles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder. There after extract of plant material was prepare by using appropriate solvent in order to follow standards norms of laboratory. The LC- MS analysis of the extract was carried out at Sophisticated analytical instrument facility (SAIF), a unit of Central drug research institute Lucknow (U.P.). Here 1mg/ml sample of extract run through HPLC- MS as per following conditions.

3-HPLC-MS conditions-

HPLC-MS analyses were carried out on a Waters Alliance 2695 HPLC system interfaced with TQD triple Quadra pole mass spectrometer (Waters Corporation, Milford, Massachusetts, US) and electro spray ionization (ESI) source.

4-Chromatographic conditions-

Chromatographic separation of the extract was carried out on ACCUCORE C_{18} (150×4.6mm, 2.6µm) at 35°C using Waters Alliance 2695 HPLC. Mobile phases were made up of 0.1% formic acid/water (A) and acetonitrile (B) and used at a flow rate of 1 µL/min using injection volume 15 µL. Gradient programme was optimized as follows: 0–1 min**,** 95–95% B; 1–10 min, 95-70% B; 10-16 min, 70-40% B; 16-24 min, 40-20% B, 24-32 min, 20-20% B, 32-35 min, 20-95% B, finally mobile phase B was maintained at 95% for the last 5 min. The system running status was automatic fixed with running time 40 minutes.

5- Mass spectrometric conditions-

Mass spectrometric analysis was performed on HPLC/TQD triple mass spectrometer in positive electro spray ionization and negative ionization modes. The operating parameters were optimized as follows: capillary voltage (VCap), 3.5kV; Cone (V), 54, extractor voltage 3 V; RF, 3 V. The source temperature and desolvation temperature optimized at 120ºC and 350 ºC, respectively. Cone gas flow, desolvation gas flow and collision gas flow were set at 30, 350 and .17 L/h, respectively. The mass range was set from *m/z* 150-2000 for analysis.

6- Interview of tribal people for Ethnobotanical /Ethno medicinal importance-

In order to know about ethno botanical importance of this plant we have performed interviews of some local inhabitants (**People of Kol Tribe**)/ vaidas of certain villages of district Chitrakoot Uttar Pradesh, India in order to collect information about various Ethno botanical/ethno medicinal importance of *A vasica* (Vasaka) as per their experience or knowledge that they gain from their ancestors. As we all of us know that various traditional knowledge/ experience of a particular community is orally transferred from their parents to descendant during generation to generation as the part of their cultural heritage.

Photo-A, Interview with people of village- Jhilang, Bahilpurwa, Chitrakoot

Photo-B, Sample in container (Leaf powder)

Results and Discussion-

1-Phytochemical analysis

The comparative base peak chromatograms (BPCs) in negative and positive mode from the extract of *A vasica* is presented in Fig. 2. There are chromatographic and mass spectrometric conditions were optimized to obtain maximum signal intensity. The comparative analysis of the extract showed that the positive ionization mode was more sensitive than negative ionization mode. Therefore, the positive ionization mode found satisfactory for alkaloids and negative ionization for flavonoids. Total sixteen compounds have been traced in our phytochemical analysis of *A vasica* including eleven alkaloids and five flavonoids were tentatively identified based on chromatographic and mass spectrometric conditions along with literature survey matching (Table 1).

2-Ethnobotanical perception

From ethnobotanical survey/ interviews it can be concluded that *A vasica* possess bronchodilator quality. That is why traditionally vasaka plant has been utilized from centuries by indigenous people to treat various ailments like asthma, cold and cough, arthritis, eczema etc. In Ayurveda, it is mentioned that vasaka has tikta, kashaya, laghu, seta and virya properties (Singh SK et. al., 2021). So it may help to treat primarily respiratory and heart diseases. Leaves extract is used traditionally by local inhabitants for the betterment of cough, asthma, bronchitis in composition with honey. They also used *A vasica* as hepatoprotective as well as for the treatment of tuberculosis & as a uterine tonic. People also told us that they used leaves of this plant in order to ripening some unripe fruits like bananas from generation to generation.

Table-1 List of identified alkaloids and flavonoides in crude extract of *Adhatoda vasica* leaves by LC-MS in positive and negative ionization mode

S. No.	Alkaloids	Mol. Formula	*m/z* $[M+H]^+$	Reference
1.	Vasicine	$C_{11}H_{12}N_2O$	189	Narasimha ji et al., (2023)
2.	Vasicinone	$C_{11}H_{10}N_2O_2$	203	Narasimha ji et al., (2023)
3.	Vasicinolone	$C_{11}H_{10}N_2O_3$	219	Singh et al., (2015a)
4.	Adhavasicinone	$C_{12}H_{12}N_2O_3$	233	Singh et al., (2015a)
5.	5-methoxy vasicinol	$C_{12}H_{14}N_2O_3$	235	Singh et al., (2015a)
6.	Vasicoline	$C_{19}H_{21}N_3$	292	Singh et al., (2015a)
7.	Vasicolinone	$C_{19}H_{19}N_3O$	306	Singh et al., (2015a)
8.	Methoxy vasicoline	$C_{20}H_{23}N_3O$	322	Singh et al., (2015a)
9.	Adhatodine	$C_{20}H_{21}N_3O_2$	336	Singh et al., (2015a)
10.	Anisotine	$C_{20}H_{19}N_3O_3$	350	Singh et al., (2015a)
11.	Methoxy vasnetine	$C_{20}H_{19}N_3O_4$	366	Singh et al. ,(2015a)
	Flavonoids		*m/z* $[M+H]^-$	
12.	Apigenin-6,8-di-*C*-arabinoside	$C_{25}H_{26}O_{13}$	533	Singh et al., (2015b)
13.	Apigenin-6-*C*-glucoside-8-*C*-arabinoside	$C_{26}H_{28}O_{14}$	563	Singh et al., (2015b)
14.	Luteolin-6-*C*-glucoside-8-*C*-arabinoside	$C_{26}H_{28}O_{15}$	579	Singh et al., (2015b)
15.	Kaempferol-3-*O*-rutinoside	$C_{27}H_{30}O_{15}$	593	Singh et al., (2015b)
16.	Quercetin-3-*O*-rutinoside	$C_{27}H_{30}O_{16}$	609	Singh et al., (2015b)

Table-2 Ethnobotanical/ Ethno medicinal uses of different parts of *A vasica*

S. No.	Parts of Plant	Disease/ Disorder
1	Roots	Gonorrhea
2	Flowers	Jaundice and eye disorder
3	Leaves	In respiratory disorders like bronchitis, expectorant, asthma and antitussive. These are also used as antiseptic, anthelmintic & in dysentery or diarrhea.
4	Entire plant	Use as beds to ripened the unripe fruits like banana

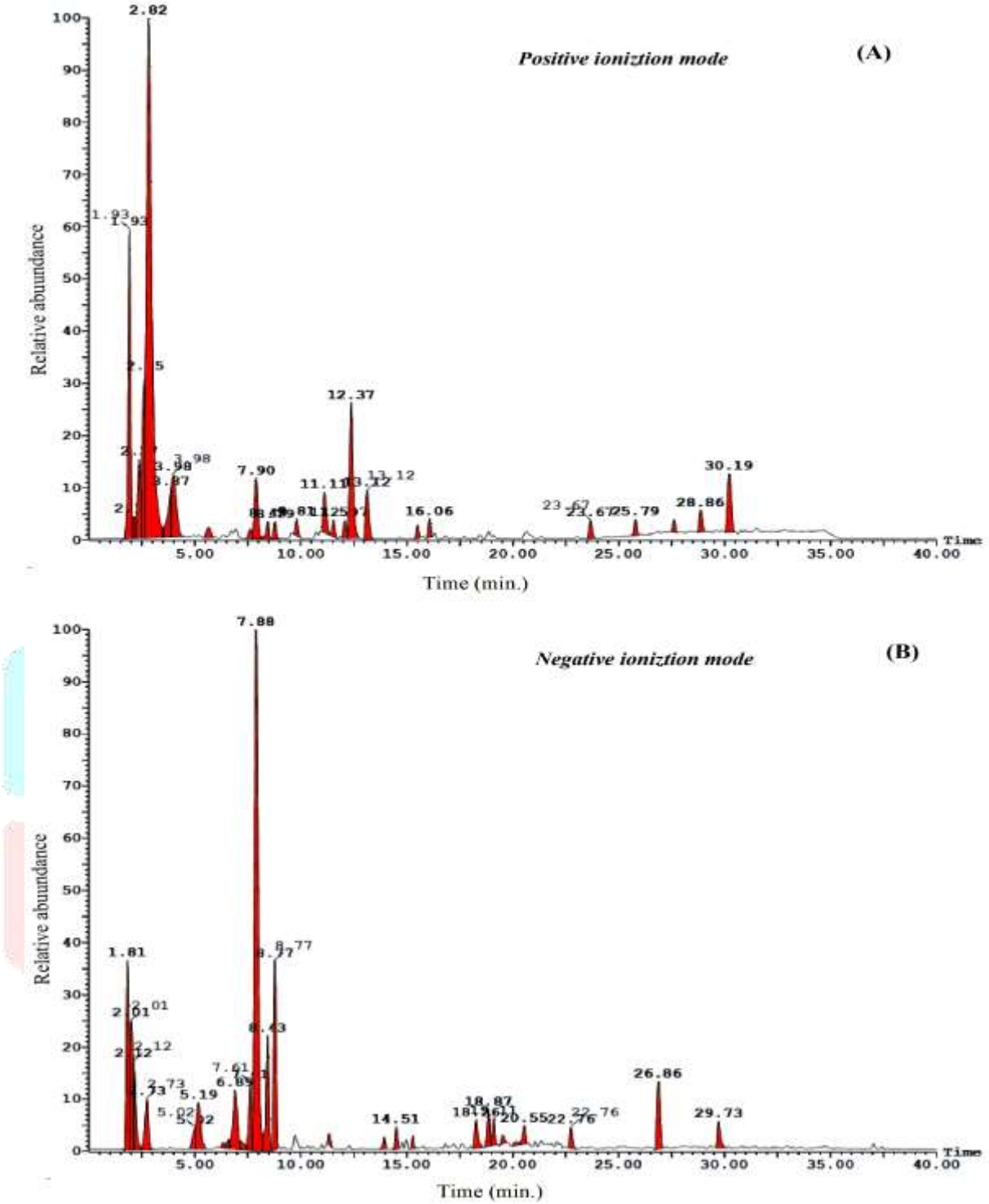

Fig. 2 Base peak chromatograms in positive (A) and negative (B) ionization mode from crude extract of *Adhatoda vasica* leaves

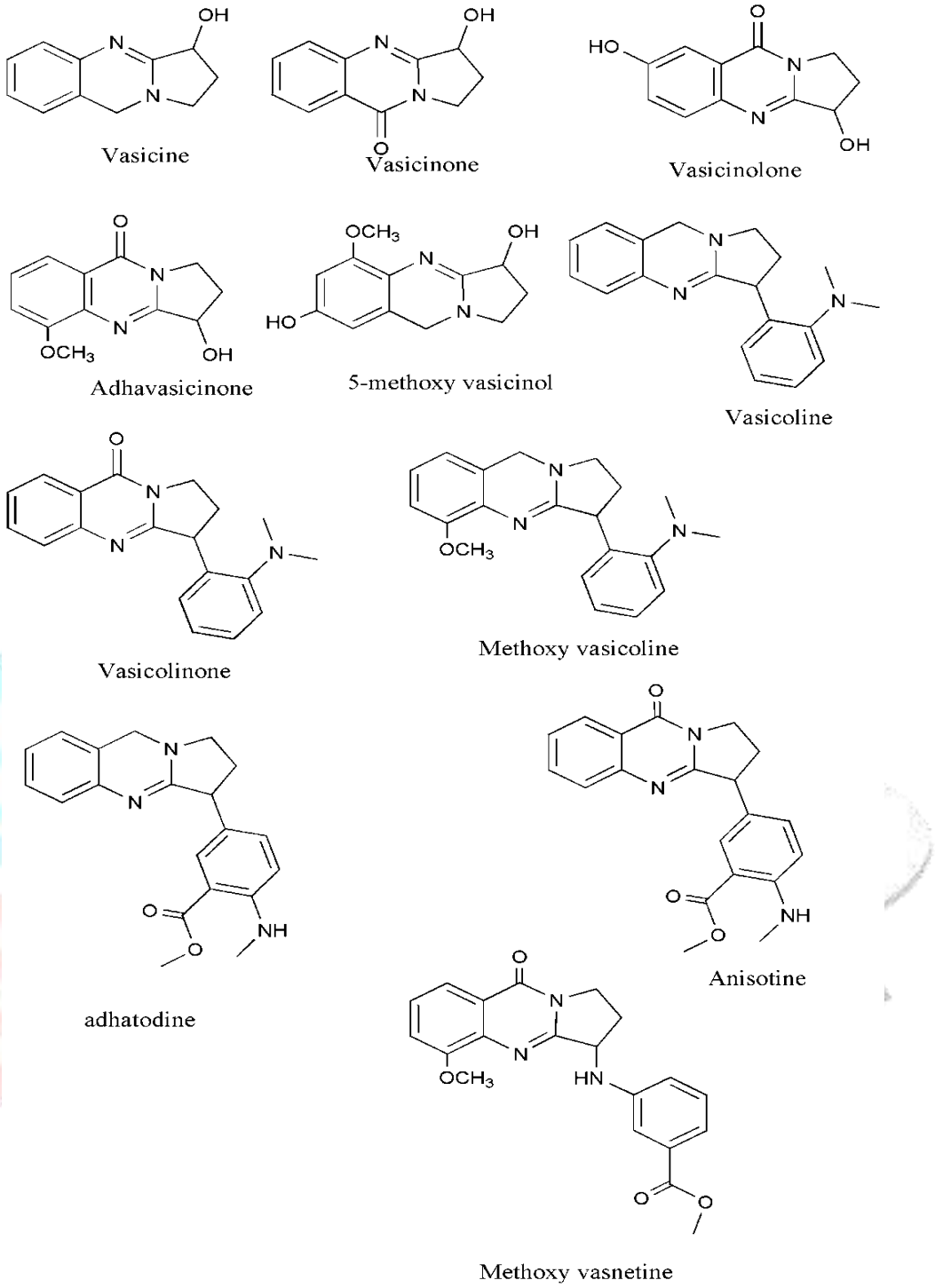

Fig. 3 Structure of identified alkaloids in positive ESI ionization mode from crude extract of *Adhatoda vasica* leaves

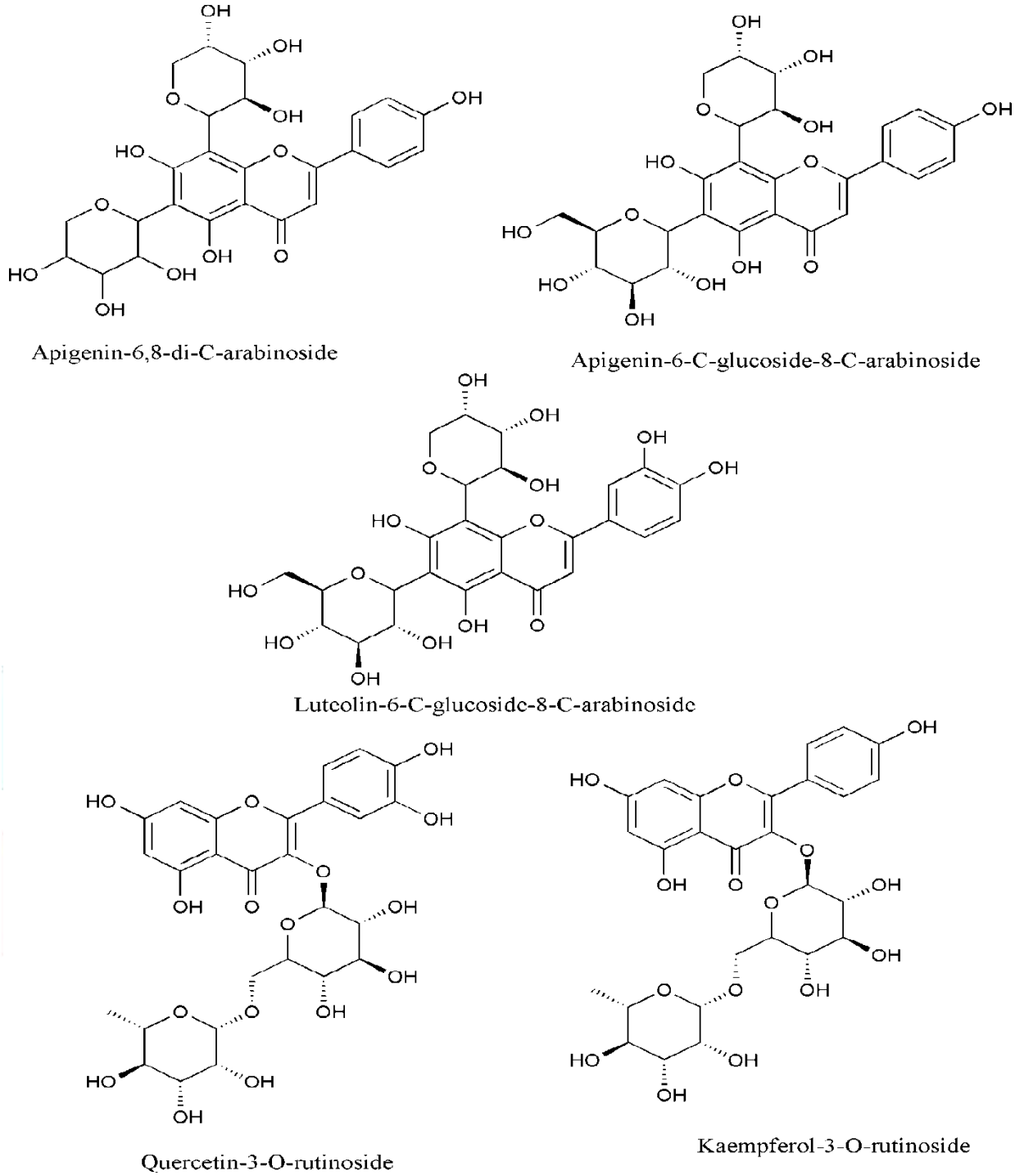

Fig. 4 Structure of identified flavonoides in negative ESI ionization mode from crude extract of *Adhatoda vasica* leaves

Conclusion-

So from this study it can be suggested that vasaka plant is highly rich in various bioactive compounds like alkaloids and flavonoids. Here also in our study 11 alkaloids and 5 flavonoids have been identified from the leaves extract of the vasaka plant through LC-MS technique. These alkaloids and flavonoids make this medicinal plant unique to cure various human diseases like cough, bronchitis, asthma etc. This plant is used in Ayurveda as well as Unani medicinal systems since prehistoric time. Ethnobotanical (folklore)) studies

revealed that this plant is beneficial to human beings especially in various respiratory diseases like cold, cough, asthma and bronchitis. During this study it also seen that many local people of district Chitrakoot used the leaves of this plant for ripening the unripe fruits. This study may surely provide a backbone for the development of a natural product having very least side effects than the synthetic or chemical compound. It will also promote to the researcher in agro industries that basically work on herbal products. The future perspective involves better resolution with the different bio active compounds isolated from the *A vasica*.

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Conflict of Interest-

Authors declared no conflict of interest.

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