

Pharmaceutical evaluation of Graphirine syrup formulated from some common indigenous herbs of Pakistan

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Abstract: Since ancient times humans have relied on traditional medicine and herbal formulation for the cure of various diseases and disorders. Globally, interest in use of herbal medicine has increased; as drawbacks of modern medicine and have started getting more prominent. Most of the traditional system of medicine; all in their formulations depends on plants. Traditional herbal cough products have considered to be mild, nontoxic, non-sedative and even harmless. As Syrup is commonly taken for the relief of cough; we aimed to formulate a cough syrup (Graphirine) from some common indigenous herbs; *Adhatoda vasica*, *Piper longum* and *Rosa Damasena* and evaluate its physicochemical parameters along with the changes in accelerated stability testing. Preformulation studies, i.e. moisture content, ash, water and ethanol extractive values of all three herbs were defined. Physicochemical factors, quantitative spectral measurement, FTIR spectrum for identification of compounds; along with accelerated stability and possible microbial fungal growth; were also checked for the formulated syrup. Each and every parameter complied with the specifications.

Keywords: Graphirine syrup, *Adhatoda vasica*, quantitative spectral measurement, FTIR, microbial fungal growth.

INTRODUCTION

Plants and plant derived products are the most sought after products in the recent years; to be used as a medicinal, nutraceutical or cosmetic agent. With the passage of time; their availability is increasing drastically; in health food shops and pharmacies. According to World Health Organization (WHO), herbs are still being used in traditional medicine and as primary health care need, by more than 80% of the world population. Herbs have obtained wide acceptability as medicinal agents for cough remedies, diabetes, liver and lung diseases, and as a memory enhancer and immunity developer agent.

Cough is a defensive mechanism necessary for respiratory tract which doesn't allow it to be blocked by foreign particles; and clear the pathways. It can be either productive, i.e. producing mucus usually with expectoration, or nonproductive (dry). That's why, antitussive agent such as dextromethorphan or codeine are normally used; but there are many side effects of using cough syrups including nausea, diarrhea, dizziness, headaches and drowsiness. In case of pregnancy, heart disease, asthma, glaucoma or enlarged prostate; special care is required with the usage of such medications. They could cause allergies in certain individuals; at the same time could lead to habitual intake due to the presence of certain ingredients. Not recommended for children as young as and in rare cases have caused death because of accidental overdose (Dealleaume *et al.*, 2009).

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Due to all these drawbacks along with high cost of western medicine, more and more people have started using herbal cough medications in the recent years. Currently the herbal medicines are highly in demand (Zakir Hussain and Sumer Singh, 2018). Moreover use of herbal medicine becoming popular due to adverse effects of conventional drugs. (WHO Expert Committee, 2003, 2005, 2007; Ahn, K, 2017). In many countries, there is little regulation of traditional medicine, but the World Health Organization establishes a network to encourage safe and rational usage of traditional medicines (Soni *et al.*, 2008; Committee on Herbal Medicinal Products, 2005, Rev. 2).

Traditional herbs and herbal cough products have been considered to be mild, nontoxic, non-sedative and even harmless. Cough Syrup, a liquid dosage form, usually given to individuals who can't swallow solid dosage form or children because of ease of administration (Chang *et al.*, 2008; Kumar *et al.*, 2013). Different herbal syrups are available in Pakistan (such as Ezicof, Linkus, Banafsha Shehtoot, Toot Siah, Surficol, Coferb, Suduri, Prospan, Cofloz, etc). Mostly content ingredients like: *Glycyrrhiza glabra*, *Hyssopus officinalis*, *Piper longum*, *Zingiber officinale*, *Hedera helix*, *Thymus vulgaris* and *Primula veris*, *Althea officinalis*, etc.

In this study, an effective herbal cough syrup is formulated and evaluated on standard pharmaceutical parameters; named Graphirine contained. *Adhatoda vasica*, *Piper longum* and *Rosa damasena* which are

frequently available in Pakistan, India and Asian region. *Adhatoda vasica* (*Acanthaceae*); is a well-known herb in indigenous systems of medicine; particularly for treating cold, cough, asthma, chronic bronchitis (Bagchi *et al.*, 2003; Dilnawaz and Irfan, 2003), tuberculosis and another lung and bronchiole disorders (Ashish *et al.*, 2009; Kumar *et al.*, 2010), along with antibacterial and anti-inflammatory properties (Chakraborty and Brantner, 2001; Kumar *et al.*, 2010). *Piper longum* (*Piperaceae*) has effects on relaxation of muscles tension, inhibitory activities on prostaglandin and leukotrienes COX-1 inhibitory effect and thus exhibit anti-inflammatory activity (Trivedi *et al.*, 2011). Studies have also revealed its antiasthmatic and anticonvulsant properties. (Nongyao *et al.*, 2004; Pallela *et al.*, 2006). *Rosa damascena* Mill. (*Rosaceae*) is one of the most important member of rose family; also an important historic medicinal agent which possess antioxidant, anti-inflammatory, and antitussive effects (Gangwar and Ghosh, 2014; Mohammad *et al.*, 2003). Studies have proved that the combination of all three medicinal plants have very potent effects against all ailments of cough and cold (Shrivastava *et al.*, 2006; Masaya *et al.*, 2007; Somesh *et al.*, 2015).

MATERIALS AND METHODS

Plant material

Plant materials were purchased from the local market and authenticated by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. The crude drugs include: dried leaves of *Adhatoda vasica* Nees, dried fruits of *Piper longum* L. and petals of *Rosa damasena* Mill. All drugs were deposited in herbarium of Department of Pharmacognosy, Pharmacy Faculty and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan (table 1).

Method of Preparation

Grinding and mixing of crude drugs

All crude drugs were grinded through Fitzpatric "Fitz mill" to a mesh size of 16 to get larger surface area for extraction. Further grinding was done by Tarrington house "Dry Mill". Grounded powder of *Adhatoda vasica* 75gm, *Piper longum* 150gm, *Rosa damasena* 75gm was added to 3000 ml of purified water; it was then boiled till one fourth volume of solution left; cooled; filtered and finally used for the syrup formulation.

Paraben solution

6 gm parabens powder was dissolved in 300 ml of boiling purified water in separate stainless-steel container, stirred continuously until clear solution obtained.

Preparation of simple syrup

2550 gm of Sucrose is weighed and added to purified water to make total mass of 3000gm in 10-liter stainless

steel pressure vessel. Heated until dissolved with occasional stirring. (BP)

Preparation of final herbal syrup

Extract of herbs and parabens solution added to sugar syrup in stainless steel pressure vessel, heated with occasional stirring, until boiled. Volume was checked through dip stick (already marked and validated) and made up to 3000 ml.

Assessment of herbal syrup

Preformulation Studies

Moisture Content

About 10 gm of each drug was used to dry in Halogen moisture analyzer HG53 at a drying Temperature 90°C (WHO, 1998; 2007; EMA, 2011). Formula used was:

$$\frac{Wc - Wr \times 100}{Wc}$$

where Wc = Weight of crude drug powder; Wr = Weight of crude drug after moisture removal (table 2)

Total ash value

Dry ash procedure was used at a high temperature in Carbolite Furnace Model, ELF 11/6, UK maintaining temperature at 550°C for half an hour. Water and other volatile materials were vaporized, and organic substances were burned in the presence of the oxygen and released CO₂, H₂O and N₂ (WHO, 1998; 2007; EMA, 2011).

Total Ash value was calculated using the formula:

$$\frac{\%Ash \text{ (dry basis)}}{100} = \frac{Ma}{Md} \times 100$$

Where Ma is mass of crude drugs before burning at high temperature and Md is mass of crude drugs after burning at high temperature (table 2)

Determination of water extractive value

5 gm powder of each crude drug was soaked in 100 ml of water in a closed flask and allowed to stand for 24 hours. The filtrate was taken in china dish; and then evaporated in water bath (Nanbei, China). The percentage of water-soluble extractives was calculated with reference to air dried crude drug. (WHO, 1998; 2007; EMA, 2011).

Formula: $\frac{Ww}{Wr} = \% w/w$

Where Ww = Weight of dried filtrate containing water extracted drug; Wr = Weight of air-dried crude drug (table 2).

Determination of Ethanol Extractive Value

10 gm coarse powder of each drug macerated with 100 ml of 95% ethanol in a closed flask; shaken frequently for first 12 hours and allowed to stand for next 12 hours. Thereafter filtered, rapidly, taking care of loss of ethanol. Evaporated filtrate to dryness in a china dish in water bath (Nanbei, China). Calculated the percentage of ethanol soluble extractive with reference to the air dried crude drug (WHO, 1998; 2007; EMA, 2011).

Table 1: Ingredients of Herbal Syrup (Graphirine)

S. No.	Name of Drug	Weights	UoM*	Proportion%
01	<i>Adhatoda vasica</i>	75	G	2.5
02	<i>Piper longum</i> L.	150	G	5.0
03	<i>Rosa Damasena</i> Mill.	75	G	2.5
05	Methyl Paraben 300	5.3	G	0.18
06	Propyl Paraben 40	0.7	G	0.20
07	Sucrose	2550	G	85.0
08	Purified water	**Qs (Conductivity 1.2 µS/cm and pH 6.5.		

*unit of measure, ** quantity sufficient

Table 2: Preformulation Studies of Crude Drugs

S. No.	Test performed	Crude drugs		
		<i>Adhatoda vasica</i>	<i>Piper longum</i>	<i>Rosa damascena</i>
01	Moisture contents	7.69%	13.28%	10.23%
02	Ash Value	14.21%	4.05%	6.1%
03	Water Extractive value	16.4% w/w	13.5% w/w	10.6% w/w
04	Ethanol Extractive value	8.1% w/w	9.7% w/w	9.1% w/w

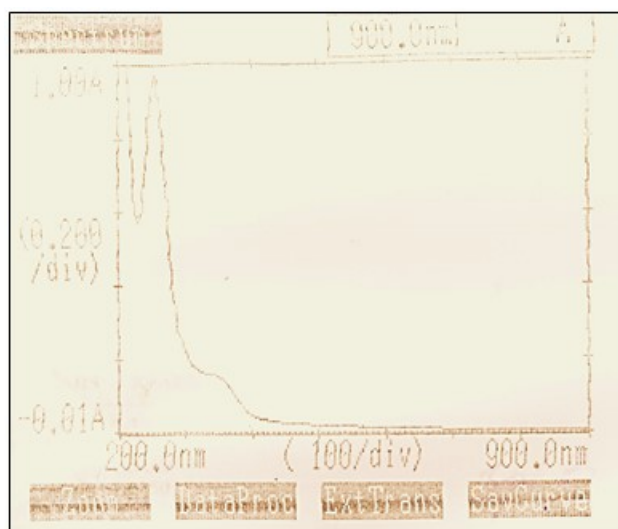


Fig. 1: UV Spectra of Purified Extracts

Formula: $We / Wr = \%w/w$

Where We = Weight of dried filtrate containing ethanol extracted drug; Wr = Weight of air-dried crude drug (table 2).

Post formulation Studies

Physicochemical Parameters

Physical appearance (i.e., colour, odour, taste), pH, Weight/ml and Specific Gravity of formulated syrup were also checked (table 3).

Organoleptic Evaluation

Syrup was also evaluated for physical appearance, taste, odour and colour (table 4)

Microbiological testing

Pour-Plate technique was employed. 9 cm diameter petri

dish was taken, poured 20mL of *Soybean Casein Digest Agar*, dried under laminar flow hood in aseptic environment, after drying 10mL of the sample syrup poured at the surface of medium *Soybean Casein Digest Agar* and incubated at 30°-35°C for 5 days to monitor growth of (TAMC) aerobic, mesophilic bacteria and aerobic fungi. Then poured 20mL of *Sabouraud Dextrose Agar*, dried under laminar flow hood in aseptic environment, after drying 10mL of the sample syrup poured at the surface of medium *Sabouraud Dextrose Agar* and incubated at 20°-5°C for 5 days to monitor growth of (TYMC) anaerobic bacteria and fungi. Three plates of each medium were prepared two for sample and one for negative control (Ph. Eur. 2018) (table 5 & 6).

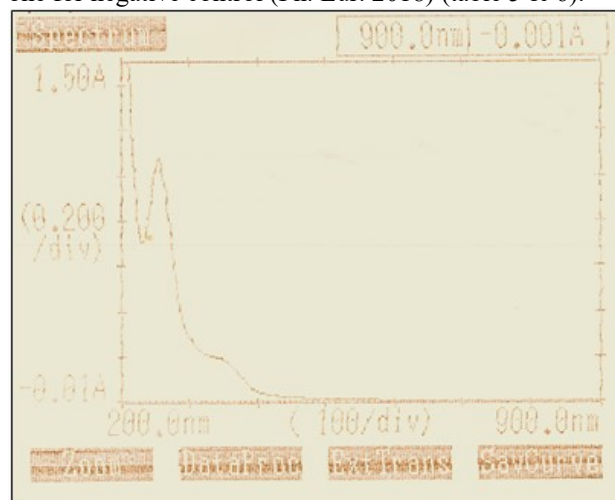


Fig. 2: UV Spectra of Reference Standard

Quantitative evaluation

The spectral evaluation of herbal extracts was done on UV-V spectrophotometer (Shimadzu UV 1601-Japan), the purified extract solution and reference standard of

Adhatoda Vasica, *Piper longum* and *Rosa damasena* Mill., were collectively dissolved in methanol at an absorption of 300 µg/mL and the spectrums of the resulting solution were taken within the range of 200-800 nm to determine the absorption maxima (λ max). The procedure was repeated thrice. λ max achieved 256nm and absorption 1.086A (fig.s 1 and 2).

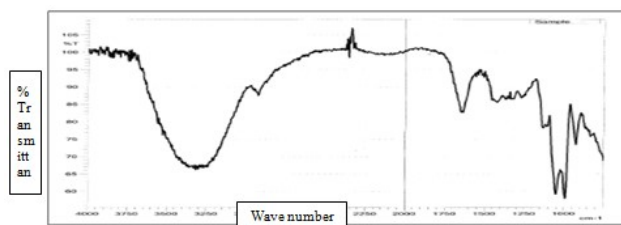


Fig. 3: FTIR Results of Purified Extracts

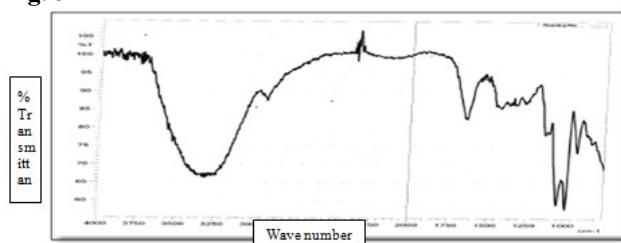


Fig. 4: FTIR Results of Reference Standard

Identifications of compounds

FTIR spectrum for identification of compounds were carried out using FT-IR (Miracle 10 Shimadzu, IR affinity 1S). The FT-IR analysis of purified extract solution and reference standard of *Adhatoda vasica*, *Piper longum*, and *Rosa damasena* was done for the detection of distinctive absorption bands of the functional groups of vasicine, piperine, and citronellol ranges from 400 to 4000 cm-1 using potassium bromide (KBr) disc (fig.3 and fig.4).

Accelerated Stability Study data

Accelerated stability conducted under Zone IVb in stability chamber “Binder GmbH” (WHO Technical Report Series, No. 953, 2009) (table 7).

Ethical approval

After successful stability studies formulated herbal cough syrup got approval from Institutional bioethics committee, university of Karachi. Certification No. IBC KU 68.

RESULTS

After performing above defines tests; we found that all pre and post formulation studies results were found within acceptable range (tables 2, 3 and 4). In microbiological evaluation, there were no growth found for six months, as shown in Tables 5 and 6. Quantitative and qualitative estimation results fall within satisfactory limits; suggesting that the product remain stable throughout the study (figs. 1-4). Similarly, no difference was seen in

accelerated stability studies and product was found stable (table 7).

Preformulation studies

Table 3: Physicochemical Parameters of Herbal Syrup

S. No.	Test	Results
1	pH Value	5.5
2	Specific Gravity (wt/ml)	1.3
3	Thermal stability	Yes
4	Degradation products	None

Post formulation studies

Table 4: Organoleptic evaluation of Herbal Syrup

S. No.	Specifications	Observations
1	Physical Appearance	Viscous liquid
2	Colour	Brownish
3	Taste	Sweet slight burning
4	Odour	Sweet aromatic

DISCUSSION

Plants play an important role in the discovery of new therapeutic agents (WHO Expert Committee, 2003, 2007). According to an evaluation of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs (Committee on Herbal Medicinal Products (HMPC) 2005, Rev. 2), the reason behind is high cost of prescription medicine and their side effects (WHO Expert Committee, 2005). Traditional herbs and herbal cough products have been considered to be mild, nontoxic, non-sedative and even harmless. Cough Syrup, a liquid dosage form, usually given to individuals who can't swallow solid dosage form or children because of ease of administration. (Chang *et al.*, 2008).

Keeping all these factors in consideration; we developed a herbal cough syrup (Graphirine) from *Adhatoda vasica*, *Piper longum* and *Rosa damasena* with pharmaceutical standard. All three herbs have been used for relief of cough both traditionally and therapeutically (Zabta *et al.*, 2009). In acute stages of bronchitis, *Adhatoda vasica* gives unfailing relief, especially where the sputum is thick and sticky (Ashish *et al.*, 2009; Kumar *et al.*, 2010). The modern medicine technology investigated its active ingredients vasicine, oxyvasicine and vasicinone as alkaloids; vasicine is the active ingredient for expelling sputum from the body. (Madhukar *et al.*, 2014; Subramanya *et al.*, 2016) In many of the cough syrups that are available, *Adhatoda vasica* has been used either as a base or as an ingredient. *Piper longum* is often use as

Table 5: Microbiological evaluation (1 to 60 days results) of Herbal Syrup

S. No.	After 1 day	Observation	After 30 days	Observation	After 60 days	Observation
1	<i>E. coli</i> (Aerobic)	Absent	<i>E. coli</i> (Aerobic)	Absent	<i>E. coli</i> (Aerobic)	Absent
2	<i>S. typhi</i> (Aerobic)	Absent	<i>S. typhi</i> (Aerobic)	Absent	<i>S. typhi</i> (Aerobic)	Absent
3	<i>P. aeruginosa</i> (Non aerobic)	Absent	<i>P. aeruginosa</i> (Non aerobic)	Absent	<i>P. aeruginosa</i> (Non aerobic)	Absent
4	<i>S. aureus</i> (Aerobic and non-aerobic)	Absent	<i>S. aureus</i> (Aerobic and non-aerobic)	Absent	<i>S. aureus</i> (Aerobic and non-aerobic)	Absent

Table 6: Microbiological evaluation (61 to 180 days results) of Herbal Syrup

S. No.	After 90 days	Observation	After 180 days	Observation
1	<i>E. coli</i> (Aerobic)	Absent	<i>E. coli</i> (Aerobic)	Absent
2	<i>S. typhi</i> (Aerobic)	Absent	<i>S. typhi</i> (Aerobic)	Absent
3	<i>P. aeruginosa</i> (Non aerobic)	Absent	<i>P. aeruginosa</i> (Non aerobic)	Absent
4	<i>S. aureus</i> (Aerobic and non-aerobic)	Absent	<i>S. aureus</i> (Aerobic and non-aerobic)	Absent

Total Aerobic Microbial Count (cfu/ml) Less than 100

Total combined Yeast and Mould Count (cfu/ml) Less than 100

Table 7: Accelerated Stability Study data of Herbal Syrup

Tests	"0" Month	1 Month	3 Months	6 Months
Temperature	40°C	40°C	40°C	40°C
RH	75%	75%	75%	75%
Physical appearance	Dark brown	Dark brown	Dark brown	Dark brown
Texture	Smooth	Smooth	Smooth	Smooth
Colour	Brown	Brown	Brown	Brown
Odour	Aromatic	Aromatic	Aromatic	Aromatic
pH value (5 to 7)	5.03	5.06	5.08	5.17
Specific Gravity	1.3	1.3	1.3	1.3
Degradation	None	None	None	None
Quantitative estimation (at 256 λ)	1.086	1.085(0.09%)	1.072(1.28%)	1.071(1.38%)

an effective drug for the treatment of Asthma and chronic bronchitis in traditional, Grecko-Arab, Ayurvedic system of medicine (Trivedi *et al.*, 2011; Pullela *et al.*, 2006) Most of the pharmacological properties of *P. nigrum* fruits are attributed to a piperidine alkaloid, piperine, which is present in the fruits in amounts of 1.7-7.4%; which act as CNS depressant, antipyretic, analgesic, anti-inflammatory, antioxidant and possess hepatoprotective activities. (Somesh *et al.*, 2015) Chemical analysis of *Rosa damascena* indicated the presence of essential oil such as citronellol, nerol, geraniol etc. Since ancient times; found effective in treating constipation, hepatitis, palpitation in traditional medicine; that is also supported by the modern pharmacological and clinical studies. (Gangwar and Ghosh 2014; Mohammad *et al.*, 2003)

After formulation, the syrup was checked for physical parameters, organoleptic characteristics, microbiological evaluation and stability studies. Preformulation studies (i.e. moisture contents, ash value, water extractive value, ethanol extractive vale) were also performed on all three drugs before the formulation of herbal syrup and all results came within acceptable range. Different post formulation tests performed on the formulated herbal

cough syrup; resulting pH (5.5) and specific gravity (1.3 gm/ml). The syrup has checked for microbiological evaluation for 1, 30, 60, 90 and 180 days; no growth of suspected organism (table. 5&6) was found; hence, syrup was found satisfactory. Accelerated stability study conducted under Zone IVb as per ICH and WHO Technical Report Series, No. 953, 2009 at 40°C and RH 75% and no differences were observed for 0 Month, 1 month, 3 months and after 6 months in any of the parameter.(Keter *et al.*, 2013; Committee on Herbal Medicinal Products, 2005). Physical appearance remained same throughout the study. Slight variation in pH from 5.03 to 5.17 occurred but remained within standard throughout the study. Quantitative and qualitative estimation results fall within $\pm 2\%$ variation from 1st sample; suggesting that the product remain stable throughout the study.

CONCLUSION

In present study, we developed an effective herbal cough syrup, which is found stable, effective and safe as per international guidelines and standards. and could be easily and efficiently used for the relief of cough in place of any

allopathic cough syrup.

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REFERENCES

- Ahn K (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports.*, **50**(3): 111-116.
- Ashish KS, Kabir A, Jasim UC and Jarifa B (2009). Characterization of an expectorant herbal basak tea prepared with *Adhatoda vasica* leaves. *Bangladesh J. Sci. Ind. Res.*, **44**(2): 211-214
- Bagchi GD, Dwivedi PD, Haider F, Singh S, Srivastava S. and Chattopadhyay (2003). Seasonal variation in vasicine content in *Adhatoda* species grown under north Indian plain conditions. *JMAPS.*, **25**: 37-40
- Chakraborty A and Brantner AH (2001). Study of alkaloids from *Adhatoda vasica* Nees and their anti-inflammatory activity. *Phytother. Res.*, **15**(6): 532-534
- Chang AB, Peake J and McElrea MS (2008). Antihistamines for prolonged non-specific cough in children. The Cochrane Database of Systematic Reviews (2): Art. No.CD005604. DOI: 10.1002/14651858.CD005604.pub3.
- Committee on Herbal Medicinal Products (HMPC) (2005). Test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products traditional herbal medicinal products. EMA/CPMP/QWP/2820, Rev. 2.
- Dealleaume L, Tweed B and Neher JO (2009). Do OTC remedies relieve cough in acute upper respiratory infections? *J. Fam. Pract.*, **58**(10): 559a-559c.
- Dilnawaz SAG and Irfan M (2003). Genetic diversity in *Adhatoda vasica* L., a remedial plant from low laying hilly areas of Azad Jammu and Kashmir Sarhad. *J. Agric.*, **19**(3): 341-346.
- EMA (2011). Guideline on specifications, test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products and, traditional herbal medicinal products. *Galantamine* (2017), Drugs.com
- Gangwar AK and Ghosh AK (2014). Medicinal uses and pharmacological activity of *Adhatoda vasica*. *Int. J. Herb. Med.*, **2**(1): 88-91.
- Keter LK, Mwikwabe NM, Mbaabu MP, Sudheer HM, Tolo FM, Dhanani P and Orwa JA (2013). Validation of Safety and Efficacy of Antitussive Herbal Formulations. *Afr. J. Pharmacol. Ther.* **2**(1): 26-31.
- Kumar KPS, Debjit B, Chiranjib Pankaj T and Rakesh K (2010). Indian traditional herbs *Adhatoda vasica* and its Medicinal application. *J. Chem. Pharm. Res.*, **2**(1): 240-245.
- Kumar M, Samarth R, Kumar M, Selvan SR, Saharan B and Kumar A (2007). Protective Effect of *Adhatoda vasica* Nees. against Radiation-Induced Damage at Cellular, Biochemical and Chromosomal Levels in Swiss Albino Mice. *Evid. Based Complement. Alternat. Med.*, **4**(3): 343-350.
- Kumar V, Shriram V and Mulla J (2013). Antibiotic resistance reversal of multiple drug resistant bacteria using *Piper longum* fruit extract. *J. Appl. Pharm. Sci.* **03**(3): 112-116.
- Madhukar G, Ennus Tajuddin Tamboli PR, Ansari SH, Abidin MZ and Sayeed A (2014). Rapid, sensitive, and validated UPLC/Q-TOF-MS method for quantitative determination of vasicine in *Adhatoda vasica* and its in vitro culture. *Pharmacogn. Mag.*, **10**(1): S198.
- Masaya I, Washita A, Nobuaki Oka, Satoko, Ohkubo, Masaki Saito and Norimichi Nakahata (2007). *Piper longum*: A constituent of *Piper longum* L., inhibits rabbit platelet aggregation as a thromboxane A2 receptor antagonist. *Eur.J.Pharmacol.*, **570**(1-3):38-42.
- Mohammad Nasser Shafeia, Hassan Rakhshandahb and Mohammad Hossain Boskabady (2003). Antitussive Effect of *Rosa damascena* in Guinea pig. *Iran J. Pharm. Res.*, **4**(2): 231-234.
- Nongyao Sawangjaroen, Kitja Sawangjaroen, Pathana Poonpanang (2004). Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *Ethnopharmacol.*, **91**(2-3): 357-360.
- Ph. Eur. (2018). Microbiological examination of non-sterile products: microbial enumeration tests. 9th Edition Chapter 2.6. 12.
- Pullela SV, Tiwari AK, Vanka US, Vummenthula A, Tatipaka HB, Dasari KR, Khan IA and Janaswamy MR (2006). HPLC assisted chemobiological standardization of α -glucosidase-I enzyme inhibitory constituents from *Piper longum* Linn: An Indian medicinal plant. *J. Ethnopharmacol.*, **108**(3): 445-449.
- Shrivastava N, Srivastava A, Banerjee A and Nivsarkar M (2006). Anti-ulcer activity of *Adhatoda vasica* Nees. *J Herb Pharmacother.*, **6**(2): 43-49.
- Somesh M, Rupali S, Swati S, Jose M and Manish M (2015). *In-vitro* Comparative Study on Antimicrobial Activity of five Extract of Few Citrus Fruit: Peel & Pulp vs Gentamicin. *J. Agri-Food & Appl. Sci.*, **9**(1): 165-173.
- Soni S, Anandjiwala S, Patel G and Rajani M (2008). Validation of different methods of preparation of *Adhatoda vasica* leaf juice by quantification of total alkaloids and vasicine. *Indian J. Pharm. Sci.*, **70**(1): 36.
- Subramanya MD, Pai, SR, Ankad, GM Hegde, HV, Roy S and Hoti SL (2016). Simultaneous determination of vasicine and vasicinone by High-performance liquid chromatography in roots of eight *Sida* species. *Ayu.*, **37**(2): 135.

- Trivedi MN, Khemani A, Vachhani UD, Shah CP and Santani DD (2011). Pharmacognostic, phytochemical analysis and antimicrobial activity of two Piper species. *Pharmacie Globale*, 7(05): 1-4.
- WHO Expert Committee (2003) on Specifications for Pharmaceutical Preparations. Thirty-seventh report. Geneva, World Health Organization, (WHO Technical Report Series, No.908, Annex 4(37): 1-72.
- WHO Expert Committee (2007). On WHO guidelines on good manufacturing practices (GMP) for herbal medicines.
- WHO Expert Committee on Specifications for Pharmaceutical Preparations and World Health Organization, (2005). WHO Expert Committee on Specifications for Pharmaceutical Preparations: Thirty-ninth Report (Vol. 39). World Health Organization.
- World Health Organization (2009). Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Technical Report Series, 953: 87-123.
- Zabta KS, Irfan K, Shamma N and Altaf H (2009). Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. *Afr. J. Biot.*, 8(24): 7082-7086.
- Zakir Hussain Khanday and Sumer Singh (2018). Important medicinal and aromatic plants of Shopian district of Jammu and Kashmir, with emphasis on their ethnobotanical importance. *IJARSE*, 738-743.