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PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENING AND FLUORESCENCE ANALYSIS OF METHANOLIC LEAF EXTRACT OF *ARTEMISIA ABSINTHIUM*

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ABSTRACT
Aim of the present chemical examination is to investigate the preliminary phytochemical screening and fluorescence analysis of the methanolic leaf extract of the plant *Artemisia absinthium* (MLEAA). The species *Artemisia absinthium* Linn (Wormwood) is a perennial shrub that belongs to the family Asteraceae, is naturally distributed in Kashmir valley and also having global distribution from Europe to North Asia, used as main ingredient in the liquor absinthe drink. Indian conventional medicinal entities like Ayurveda, Siddha and Unani use this plant parts for treating several diseases. The main active constituents found in *Artemisia absinthium* are bitter compounds and essential oil. The analysis of the crude drug has shown some important phytochemical constituents that are well known for their important pharmacological action. The results of preliminary phytochemical screening and fluorescence of *Artemisia absinthium* are very helpful in assurance of quality and purity of the marketed crude drug and its formulation. The recognized phytoconstituents of this qualitative evaluation will be helpful for future pharmacological examination of this plant species *Artemisia absinthium* Linn.

KEYWORDS: *Artemisia absinthium*, absinthe, Asteraceae, fluorescence, Kashmir.

INTRODUCTION
Artemisia absinthium is an aromatic, bitter, frutescent and perennial plant with fibrous roots. It is indigenous plant of Kashmir region in India.[12, 22] Mostly all parts of the plant are useful -leaves, flowering top, roots and stem. They are used to treat chronic fevers, swellings, inflammation of liver, menstrual disorders, enfeebled digestions, debility, as vermifuge (flowers), tonic in intermittent fever. Wormwood oil is used externally in rheumatism. Whole plant, seeds, flowers and roots of the Worm wood are used to make herbal medicines in Ayurveda, Homeopathy, Unani, Siddha and even in Modern medicine.[17]

Artemisia absinthium has displayed osmotic stability of human erythrocytes, free-radical scavenging activity, cognitive enhancement function, neurite outgrowth function, antiprotozoal activity, antimalarial activity, antifungal activity, antihelmintic activity. *Artemisia absinthium* is having antiulcer activity, antimicrobial activity, anti-cancerous activity, hepato protective activity, intoxicating effect, anti-nemathelminthic activity, antipyretic activity, anti-oxidative stress function, antibacterial activity, antioxidant activity.[15] The major active constituents found in *Artemisia absinthium* are essential oil and bitter compounds. The composition of essential oil varies according to geographical source .Fatty acid composition of the oil, sesquiterpene lactones, volatile compounds, flavonoid compounds; Polyphenol compounds, phenolic acids, lignins, sesquiterpene, sterolic compounds.[6]

The present study deals with the preliminary phytochemical evaluation of procured material from a commercial herbal store for confirmation purpose, it as the methanolic leaf extract (MLEAA) of *Artemisia absinthium*.

MATERIAL AND METHODS
Plant material
Methanol leaf extract of *Artemisia absinthium* (dry powder) and dried leaves were purchased from Mahaks Herbal & Aromatic Agro Products, Srinagar, and Jammu & Kashmir and the voucher specimen of the plant has been be deposited in the department of biotechnology, Sigma Biosciences Research Centre, Bengaluru, Karnataka, India, a recognized research Centre of Tumkur University, Tumakuru.

Extraction procedure of MLEAA
Extraction procedure followed by the herbal company is; leaves of *Artemisia absinthium* were air dried under the

shade for a week. 500g of dried leaves were powdered, sieved with mesh and extracted with 1.5L of methanol (80%) using soxhlet apparatus at 70°C for 5 hours. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure at 60°C.

Qualitative phytochemical Screening

The qualitative chemical tests were performed for methanolic leaf extract of *Artemisia absinthium* (MLEAA) according to the methods described by Sofowora,1993; Farnsworth, 1996; Khan *et al.*, 2011; Javed *et al.*, 2012; Godghate *et al.*, 2012 [4, 5, 8, 10, 21] with some revisions.

Molisch test for carbohydrates

Small quantity of plant extract was dissolved in 5 mL of distilled water and filtered. To this filtrate solution 2-3 drops of α-naphthol was added and 1 mL of concentrated sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet coloured ring at the interface to detect the presence carbohydrates.[21]

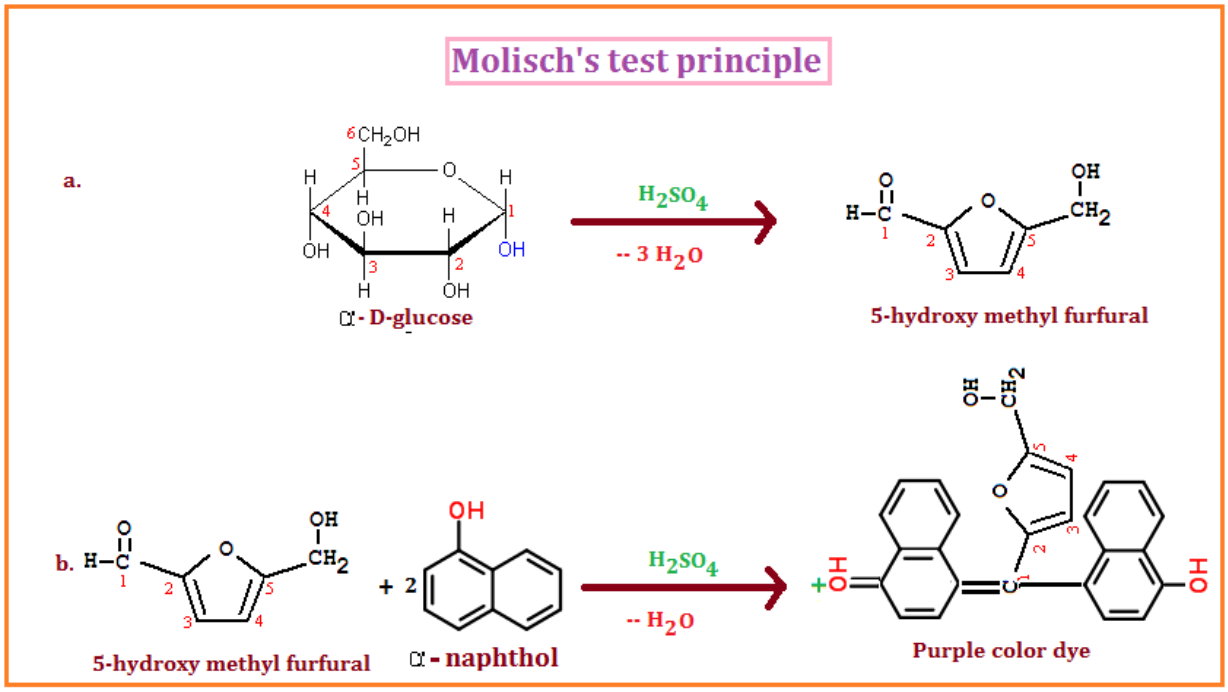

Figure 1. Principle mechanism of Molisch reagent with carbohydrates.

Xanthoproteic test for proteins

Plant extract is added with few drops of conc.HNO_3, development of yellow color indicates the presence of proteins.[5]

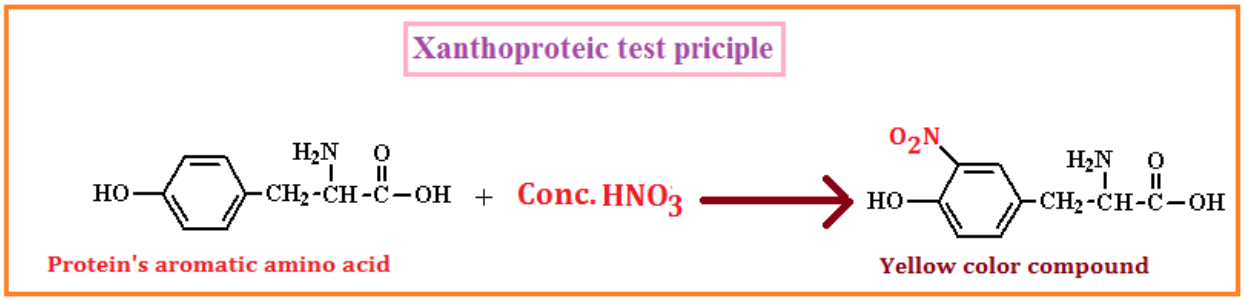

Figure 2.Principle mechanism of Xanthoproteic test for proteins.

Keller killanis test for Glycosides

Plant extract is dissolved in 2ml of ferric chloride - glacial acetic acid solution; then it is under layered with 1ml of concentrated sulphuric acid. Appearance of a brown ring at the interface indicates the presence of glycosides.[5]

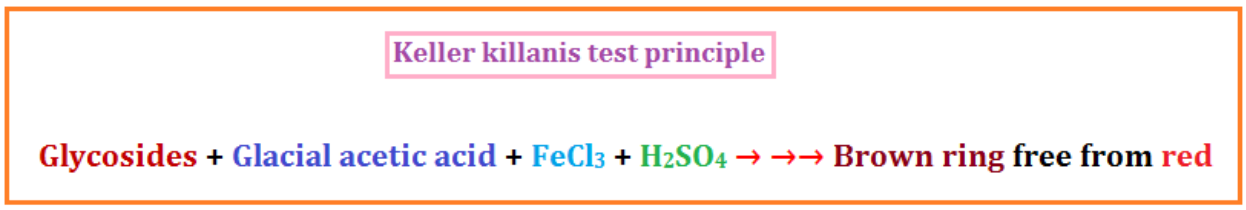

Figure 3.Principle mechanism of Keller killanis test for Glycosides.

Foam test for Saponins

Plant extract- 0.5 g in a test tube is added with little measure of water, shaken vigorously for a minute and watched for the development of rich foam, which is stable and persistent for over ten minutes, indicates the presence of Saponins.[4, 5, 8, 10]

$FeCl_3$ test for phenolic Compounds

To the 2ml of 1% aqueous plant extract 2 to 3 drops of 5% ferric chloride solution. Appearance of deep violet color or black color indicates the presence of phenolic compounds.[8]

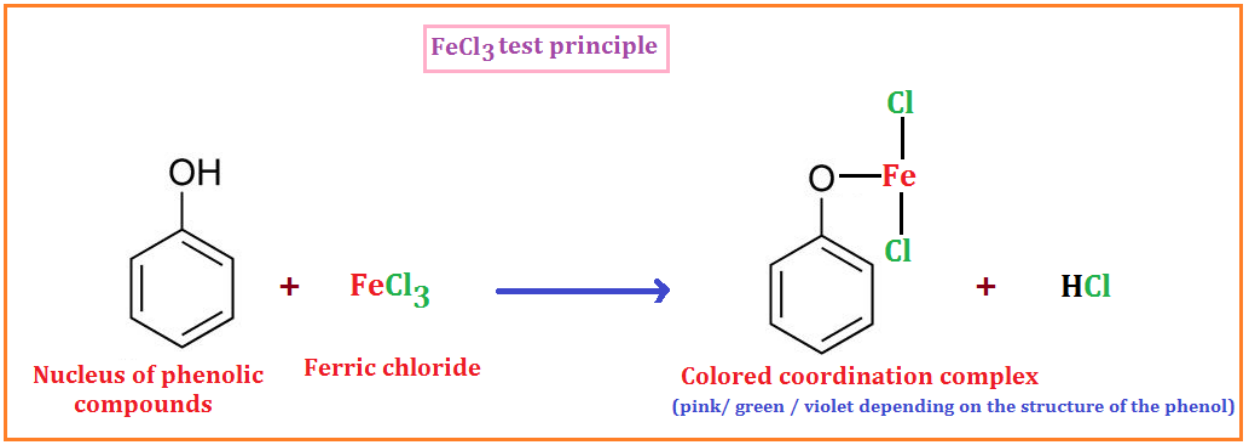

Figure 4.Principle mechanism of $FeCl_3$ test for phenolic Compounds.

Salkowski's test for phytosterols

Chloroform was added to the plant extract, mixed and filtered. Then the filtrate was treated with few drops of concentrated H_2SO_4, stirred and allowed for standing, development of golden red color indicates the presence of phytosterols.[4]

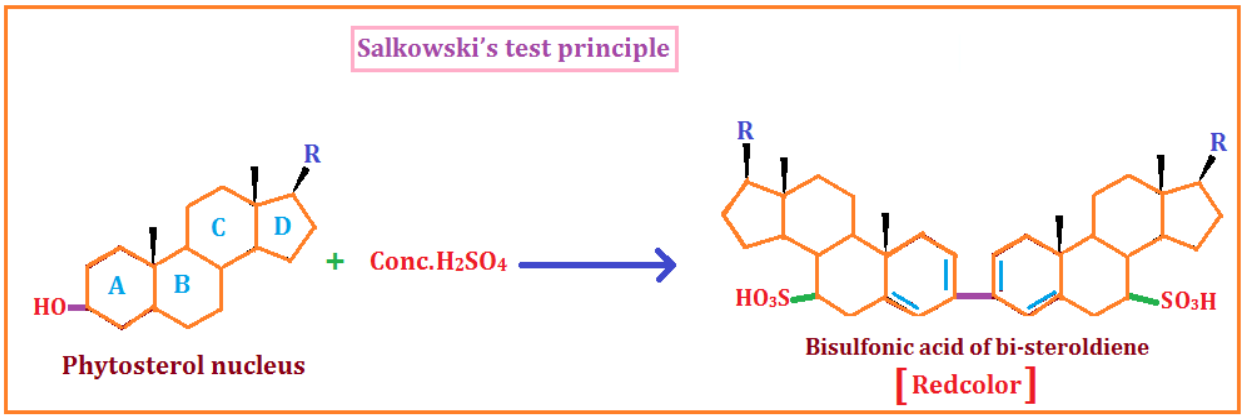

Figure 5. Principle mechanism of Salkowski's test for phenolic Compounds.

Mayer's test for Alkaloids

Mayer's test reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100 ml of water) is added to the small amount of plant extract in the test and watched for the formation of cream colored precipitate.[21]

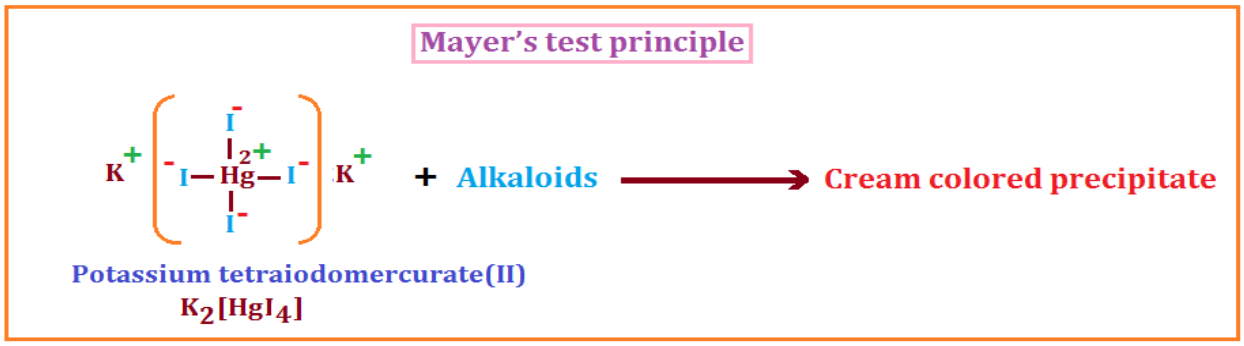

Figure 6. Principle mechanism of Mayer's test for Alkaloids.

Acetic acid test for tannins

To the small quantity of plant extract few ml of acetic acid is added and watched for the development of red color, which indicates the presence of tannins.[3]

Shinoda's test for flavonoids

In a test tube 0.5 grams of plant extract is dissolved in 5ml of 95 % ethanol, warmed and then filtered. Then few pieces of magnesium metal turnings and 5-6 drops of concentrated HCl, development of crimson red color indicate the presence of flavonoids.[4, 5, 8 and 22]

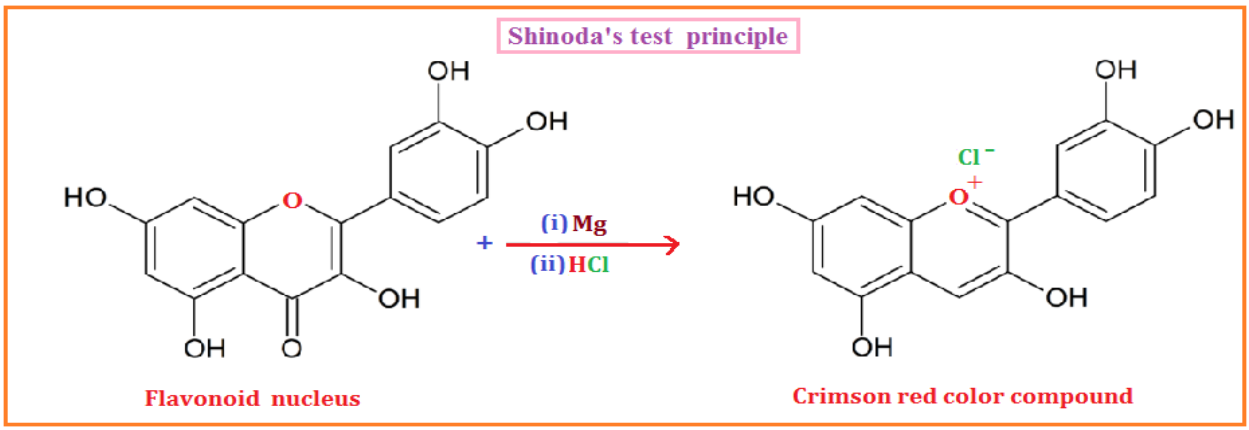

Figure 7. Principle mechanism of Shinoda's test for flavonoids.

Procedure of fluorescence analysis of MLEAA powder

A small quantity of methanolic leaf extract of *Artemisia absinthium* (MLEAA) powder was placed on a glass slide and added with 1or 2 drops of freshly prepared chemical solutions, by tilting the slide gently the components are well mixed, stand for 2 minutes and the developed color was observed and recorded under normal visible light and UV light (255nm).[7,13] Mixing combinations of different chemical solutions with MLEAA are - MLEAA powder with methanol (i.e. MLEAA powder as such), MLEAA powder with water, MLEAA powder with conc. HCl, MLEAA powder with conc. H_2SO_4, MLEAA powder with conc. HNO_3, MLEAA powder with 0.1N NaOH and MLEAA Powder with acetone are used to perform for fluorescence analysis.[11]

RESULTS

Preliminary qualitative phytochemical analysis

Preliminary phytochemical analysis of the current study has confirmed that the procured material is methanolic extract of *A. absinthium* (MLEAA) through display of absence of alkaloids and presence of the following compounds -carbohydrate, proteins, glycoside, saponins, phenolic compound's, phytosterols, tannins, flavonoids that are primary phytoconstituents of *A. absinthium* for its characteristic identification at the basic level of analysis and the data presented in the representative table.

Fluorescence analysis

The fluorescence analysis of MLEAA as such in its powdered form has displayed light green color under UV light at 255nm and pale green under visible daylight. Fluorescence data obtained due to MLEAA treatment with various chemical reagents is displayed in the representative table.

DISCUSSION

Phytochemical analysis

To guarantee the reproducible nature of plant based materials, legitimate control of beginning material is most basic requisite. To maintain such standards, preliminary chemical analysis and fluorescence record of the crude natural drugs is more important. The active chemical constituents present in the crude drugs are evaluated through qualitative phytochemical tests. Our

current results of phytochemical analysis are in concurrence with results of Ashok and Upadhyaya 2013; Rajeev *et al*., 2012; Aberham *et al*., 2010.[1, 2 and 18] Kundan and Anupam, 2010 as well as Kamel *et al*., 2015 have also reported glycosides, saponins, poly phenolic compounds and tannins as major phytochemicals of *A. absinthium* in several solvent extracts along with methanol.[9,14] Monika and Kaur, 2016 have reasoned the phytoconstituents of methanol extracts of the plant are more effectively responsible for the antimicrobial activity when compared to other extracts.[16] In a review Saiedi and Masoudi, 2017 has stated that, different therapeutic and pharmacological actions of *A. absinthium* are due to the presence of major chemicals like glycosides and polyphenols.[20]

Preliminary phytochemical analysis of MLEAA has evaluated quality in terms of authentication, adulteration of crude drugs and distinctive presentation of polar and nonpolar or phylic and phobic extractable chemical components in different solvents. Finally, chemical screening is useful for the development of the crude plant extracts for the prescribing as a standardized phyto drug.

Fluorescence analysis

The current fluorescence data displayed in the representative table by MLEAA with various chemical reagents is in concurrence with earlier studies of Ashok and Upadhyaya, 2013; Javed *et al.*, 2012.[2, 8] The fluorescent analysis of powdered plant extract plays an important role in the determination of quality and purity of the commercially purchased natural drugs in their crude form. The non-fluorescent active constituents are identified by converting them into fluorescent derivatives by treating with different chemical solutions and observing under either visible or UV light for developed coloration. Unique coloration of MLEAA upon chemical treatment with different reagents has evaluated its pharmacognostic purity, which is an important parameter for the standardization of herbal therapeutics.

Table 1. Phytochemical screening of methanolic leaf extracts *Artemisia absinthium* [MLEAA].

S. No.	Constituents	Tests	MLEAA
1	Carbohydrate	Molish's test	+
2	Proteins	Xanthoproteic test	+
3	Glycoside	Keller killanis test	+
4	Saponins	Foam test	+
5	Phenolic compunds	$FeCl_3$ test	+
6	Phytosterol	Salkowiski test	+
7	Alkaloids	Mayer's test	-
8	Tannins	Acetic acid test	+
9	Flavonoids	Shinoda's test	+

Phytochemicals of the MLEAA were shown in the table as; Presence as + and absence as **-.**

Table 02. Fluorescence analysis of MLEAA powder with various chemical reagents.

Treatment Number	Powdered plant extract	Chemical reagent	Color seen under UV light 255nm	Color seen under Visible light
1	MLEAA	As such (methanol)	Light green	Pale green
2	MLEAA	water	Brown	Light green
3	MLEAA	conc. HCl	Black	Brown
4	MLEAA	conc.H_2SO_4	Black	Brown
5	MLEAA	NaOH (0.1N)	Light yellow	Yellow
6	MLEAA	conc.HNO_3	Light green	Dark green
7	MLEAA	Acetone	Brown	Green

MLEAA: Methanolic leaf extract of *Artemisia absinthium*

CONCLUSION

Current preliminary phytochemical analysis has confirmed that the procured material is methanolic extract of *Artemisia absinthium* (MLEAA) through display the of carbohydrate, proteins, glycoside, saponins, phenolic compounds, phytosterols, tannins, flavonoids and the absence of alkaloids that are primary phytoconstituents of *Artemisia* absinthium for its characteristic identification by the basic level of analysis. The fluorescence analysis of powdered plant extract has played an important role in the determination of quality and purity of the commercially purchased natural drug of Artemisia absinthium in its crude form.

It is concluded that both preliminary phytochemical screening and fluorescence analysis of methanolic leaf extract of *Artemisia absinthium* (MLEAA) has ensured the reproducible quality, purity and proper control of the commercially purchased crude form of natural drug and confirmed the procured material is pure and containing characteristic compounds of *Artemisia absinthium.*

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REFERENCES

1. Aberham A, Cicek SS, Schneider P, Stuppner H, 2010. Analysis of Sesquiter-pene Lactones, Lignans and Flavonoids in Wormwood (Artemisia absinthium L.) Using High-Performance Liquid Chromatography (HPLC) − Mass Spec-trometry, Reversed Phase HPLC, and HPLC−Solid Phase Extraction−Nuclear Magnetic Resonance. *J. Agr. Food Che*., 58(20): 10817-10823.
2. Ashok PK and Upadhyaya K, 2013. Preliminary Phytochemical Screening and Physico-Chemical Parameters of Artemisia absinthium and Artemisia annua. *J. Pha. Phy*., 1(6): 229-234.
3. Dhivya and Manimegalai K, 2013. Preliminary Phytochemical Screening and GC- MS Profiling of Ethanolic Flower Extract of *Calotropis gigantea* Linn. (Apocynaceae). *J. Pha. Phy. Chem*; 2 (3): 28-32.
4. Farnsworth NR, 1996. Biological and Phytochemical screening of plants. *J. Pha. Sci*; 55: 225-276.
5. Godghate A, Sawant R and Sutar A, 2012. Phytochemical analysis of ethanolic extract of roots of *carrisa carandus linn. Ras. J. Che.*, 5(4): 456-459.
6. Goud B J, Dwarakanath V, Swamy B K C, 2015. A Review on History, Controversy, Traditional Use, Ethnobotany, Phytochemistry and Pharmacology of *Artemisia Absinthium* Linn, *Int. J. Adv. Res. Eng. App.* Sci., 4(5): 77-107.
7. Gupta MK, Sharma PK, Ansari SH, Lagarkha R, 2006. Pharmacognostical evaluation of Grewia asiatica fruits. *Int J Pla.Sci*; 1(2): 249-251.
8. Javed A, Showkat M R, Naquvi K J, 2012. Preliminary pharmacognostical standardization of aerial parts of *Artemisia absinthium linn*. *Int. Res. J. Pha*., 3(1): 217-219.
9. Kamel M, Salem N, Bachrouch O, Bousselmi S, Tammar S, Alfaify A, Khaldoun A, Sane, Ammar WB, Azeiz S, Brahim AH, Hammami M, Selmi S, Limam F, and Marzouk B, 2015. Chemical composition and antioxidant and antimicrobial activities of Wormwood (Artemisia absinthium L.) Essential Oils and Phenolics. *J. Che.*, 15: 1-12.
10. Khan F A, Hussain I, Farooq S, Ahmad M, Arif M and Rehman I U, 2011. Phytochemical Screening of Some Pakistanian Medicinal Plants. *Mid-Eas J. Sci. Res*, 8(3): 575-578.
11. Khandelwal K R. 2000. Practical Pharmacognosy technique and experiments.2nd ed. Pune: *Nirali Prakasan*, 149-156.
12. Kirtikar K.R. and Basu B D, 2001. Indian Medicinal Plants. 2nd Edition, *Oriental Enterprises*, Uttaranchal, Volume 8, 2604.
13. Kokashi CJ, Kokashi RJ, Sharma M, 1958. Fluorescence of powdered vegetable drugs in ultra-violet radiation. *J. Ame. Pha. Ass*; 47: 715-717.
14. Kundan SB and Anupam S. 2010. Phytochemical and pharmacological potential of Artemisia absinthium Linn, and Artemisia asiatica Nakai: A Review. *J. Pha Res*., 3(2): 325-328.
15. Lutz DL, 2008. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. *Phy. Che.*, 69(8): 1732-1738.
16. Monika and Kaur G. 2016. Phytochemical screening and antimicrobial activity of Artemisia absinthium. *Int. J. Res. Rev.*, 3(3): 69-73.
17. Nikhat S, Ahmad S, Akhtar J and Jamil S 2013. Phytochemical and ethnopharmacological perspective of Afsantin (Artemisia absinthium Linn.) *Ann. Phy. med*; 2(2): 105-109.
18. Rajeev S, Pawan K.V and Gagandeep. S. 2012. Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. *J. Int. Eth.* 1(2): 101-104.
19. Shafi S and Tabassum N, 2013. Survey on Anti-Diabetic Plants in Kashmir (India); *J. Adv. Pha. Edu. & Res.* 2013: 3(4): 306-318.
20. Saiedi M and Masoudi M, 2017. A Review study of Ethnopharmacology, Phy-tochemistry, anti-inflammatory, antioxidant and anti-microbial effect of Arte-misia absinthium. *Der. Pha. Let.* 9(4): 155-162.
21. Sofowora A, 1993. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp. 134-156.
22. Trease G E. and Evans W C, 2002. Pharmacognosy. 15th Ed. *Saunders Publishers*, London. pp. 391-393.