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PHYTOCHEMICAL AND PHARMACOGNOSTICAL SCREENING OF *JASMINUM MULTIFLORUM* (BURM. F.) AND *JASMINUM MESNYI* (HANCE) STEMS

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Keywords:

Jasminum multiflorum, *Jasminum mesnyi* standardization, Pharmacognostical, Phytochemical

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ABSTRACT: *Jasminum multiflorum* and *Jasminum mesnyi*, the ornamental shrubs belonging to family Oleaceae are widely distributed throughout India. Despite their use in the essence industry for making perfumes, oils, and creams, these species are also used in the healing wounds and ulcers, constipation, flatulence, skin diseases, rheumatism, stomatitis, diabetes, stress, antiseptic, diuretics, and gastric disturbance. The present study is designed to establish the authenticity of both species by evaluating various standardization parameters. The stems of both species were collected and dried under shade. Freehand transverse sections were taken for microscopic examination. The standardization parameters such as Moisture content, Ash values, Extractive values, Heavy metal content, Aflatoxin content, Microbial infestation and, Pesticides residue have been determined as per WHO guidelines. Results showed the *J. multiflorum* stem was greenish-grey in color, cylindrical shaped, fragile and have aromatic odor. Microscopy showed the presence of glandular multicellular trichomes, epidermis, collenchyma, endodermis, medullary rays, vessels, xylem cells, parenchyma, fibre bundle and calcium oxalate crystals. The stem of *J. mesnyi* is yellowish-green in color the surface is smooth and pubescent. The epidermis, collenchymas, endodermis, medullary rays, xylem cells, vessels, trichomes and parenchyma are also present in the microscopy. Preliminary phytochemical screening of both plants confirmed the presence of alkaloids, anthraquinone glycosides, saponins glycosides, cardiac glycosides, flavonoids, steroids, terpenoids, tannins, and phenolic compounds. The analysis of Heavy metal, Aflatoxin, microbial contamination and pesticide residual values were found within limits. The result of our study confirmed the authenticity of both the species and the parameters studied can be used in the future as a part of the monograph.

INTRODUCTION: Ayurveda is one of the most renowned traditional systems of medicine that has survived and flourished for ages to date. In the modern time, when the allopathic system of medicine has reached almost the top because of validated research and advanced techniques still herbal medicines are the choice of drug for several chronic diseases such as cancer, diabetes, arthritis, and asthma. The various species of the genus *Jasminum* are used by people for their effectiveness.

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The genus *Jasminum* belongs to the Oleaceae family having 200 species throughout the world and out of which around 40 species are native to India [1]. *Jasminum multiflorum* is also known as winter jasmine or musk jasmine; which is an ever green winner shrub with young branches dressed

with velvety pubescence. *Jasminum mesnyi*, well known as Yellow jasmine, is a perennial shrub [2, 3]. Being known for there fragrances, the flowers are used in aromatherapy for uplifting the mood. Both the species are found throughout India, in the forests of Western Ghats, sub- Himalayan tracts up to 1300-1500 meters altitude and are also cultivated as garden plant [4]. Traditionally these plants are used to treat a variety of ailments like treat anxiety, anorexia, diabetes, depression, gastric disturbance, muscular pain, nocturnal emission, oral sores, stress, ulcer, uropathy, cephalalgia, cardiac disorders, constipation, indigestion, inflammation, rheumatism, lactifuge, weakness of sight, and snake bite [5-9].

The various secondary metabolites namely alkaloids, glycosides, flavonoids, steroids, terpenoids, tannins and saponins are substantiated in different parts of the plants [5, 10, 11]. The pharmacological studies proved their efficacy in *J. multiflorum* towards antioxidant [12, 13], antimicrobial [14], antihelmintic [15], nemacitidal [16], GI [17], in cardiovascular diseases [18, 19] and effect on central nervous system [20, 21] and *J. mesnyi* revealed its wound healing potential of roots [22], antioxidant [23, 24], antiulcer [25], antihyperglycemic [26], antihelmintic [27] and antimicrobial [28] efficacy of leaves were substantiated. The main hindrance to the acceptance of herbal medicines is the lack of documentation and quality control. The establishment of pharmacognostic standards for the traditional plants is a prerequisite for its detailed phytochemical and pharmacological investigation. The present study was done for establishing pharmacognostic and physicochemical standards of the plant stem as per WHO Guidelines which will further help in drug identification as well as in detecting/establishing adulteration.

MATERIALS AND METHODS:

Plant Collection and Authentication: *J. multiflorum* and *J. mesnyi* stems were collected from the Botanical Garden of Hindu College of Pharmacy, Sonipat. The plants were authenticated by Dr. Sunita Garg (Emeritus Scientist, CSIR-NISCAIR, and New Delhi, India). The voucher specimen(NISCAIR/RHMD/CONSULT/2018/3296-97-1 and 3296-97-2 for *J. multiflorum*, *J. mesnyi* respectively) was deposited in the Department of Pharmacognosy, Hindu College of Pharmacy, Sonepat for future reference. The plant materials were dried under shade by placing in a single layer and coarsely powdered by hand mixer and pass through sieve no #60.

Macroscopy: Untreated powdered samples were observed under daylight and the color of samples was recorded. The powder was rubbed slowly between fingers and odor was examined. Tastes of the powder were also observed. The surface was examined by softly touching it [29].

Microscopy: The thin transverse sections of both fresh stems were cut with the help of a sharp blade and cleared with chloral hydrate solution. Freehand sections was stained with phloroglucinol, ruthenium red, safranin and hydrochloric acid and further mounted in glycerine and covered with coverslip. For powder microscopy, the powder of stems was passed through sieve no # 60 and taken on glass slides. The same procedure was followed as mentioned above to prepare the slides. The slides were observed under a compound microscope and photographs were taken using Labomed ATC- 2000 microscope attached with camera [29-32].

Qualitative Analysis: The procedure recommended by Indian Pharmacopoeia was used for determining the total ash, water-soluble ash, acid insoluble ash, water-soluble extractive, ethanol- soluble extractive, loss on drying, swelling index, crude fibre content and foaming index [29-32], where as aflatoxin content, microbial contamination, heavy metal analysis and pesticidal residues were evaluated according to WHO guidelines [32]. A fluorescence study was performed as per standard procedure [29, 33].

Preliminary Phytochemical Screening: For the preliminary phytochemical screening, extracts of both species were prepared according to the polarity of the solvents (petroleum ether, chloroform, ethanol and aqueous) as per the standard procedure. The preliminary phytochemical screenings of extracts were performed according to standard procedure [29, 33].

RESULTS AND DISCUSSION:

Macroscopy: *J. multiflorum* of outer bark is thin and greenish-grey in color. The stem is buff colored with the central brown part.

The outer surface is rough, longitudinal wrinkles are present with alternate nodes and yellowish spots are present on the bark. Fracture is easy and uneven. The stem is thin and cylindrical in shape as shown in **Fig. 1**. It is bitter in taste and odor is aromatic. The stem of *J. mesnyi*is yellowish-green in color, the surface is smooth and pubescent. The nodes are present in the opposite positions; internodes are uniform about 4 to 5cm as shown in **Fig. 2**. The internal color of the stem is brown. The dried stem is woody and the fracture is smooth and easy to smash.

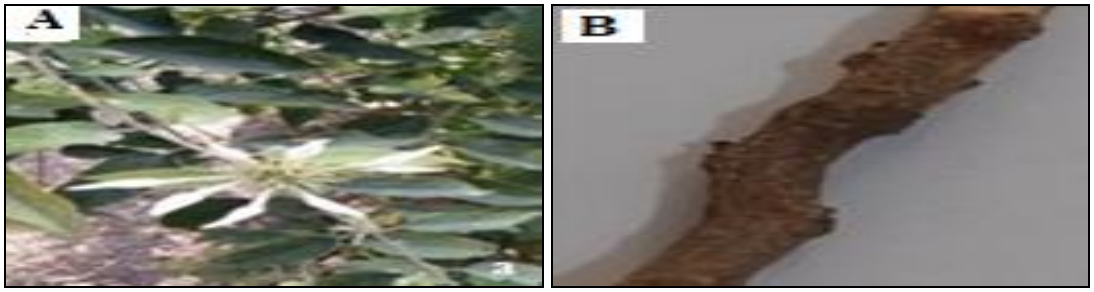

FIG. 1: (A) *J. MULTIFLORUM* PLANT SHOWING LE AVES, BUD, FLOWER AND STEM; (B) DRIED STEM

FIG. 2: (A) *J. MESNYI* PLANT SHOWING LEAVES FLOWER AND STEM; (B) DRIED STEM

Microscopy: Transverse section of *J. multiflorum* stem showed the presence of non-glandular, multicellular trichomes covering the epidermis. Collenchyma lies beneath the epidermis layer followed by endodermis. Medullary rays, vessels, xylem cells, fibre bundle, and parenchyma were observed under the endodermis layer. Vessels are mostly isolated or present in a group of two to four. Some cells were observed with calcium oxalate crystals as shown in **Fig. 3, 4.** The transverse section of the *J. mesnyi* stem showed a quadrangular shape showing entire epidermis. Under the epidermis, the collenchyma layer is presently followed by endodermis. Medullary rays, xylem cells, and vessels and parenchyma were observed under the endodermis layer. Vessels are mostly isolated and unicellular trichomes were presentas shown in **Fig. 5, 6.**

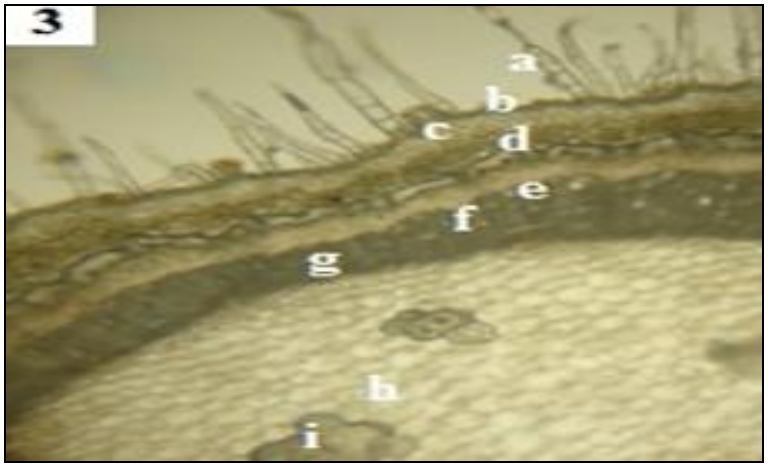

FIG. 3: REPRESENTATATIVE PHOTOMICROGRAPH OF TRANSVERSE SECTION STEM *J. MULTIFLORUM* AT 10 X SHOWING (A) AGLANDULAR MULTICELLULAR TRICHOMES; (B) EPIDERMIS; (C) COLLENCHYMAS; (D) ENDODERMIS; (E) VESSELS; (F) XYLEM CELLS; (G) MEDULLARY RAYS; (H) PARENCHYMA AND (I) FIBRE BUNDLE

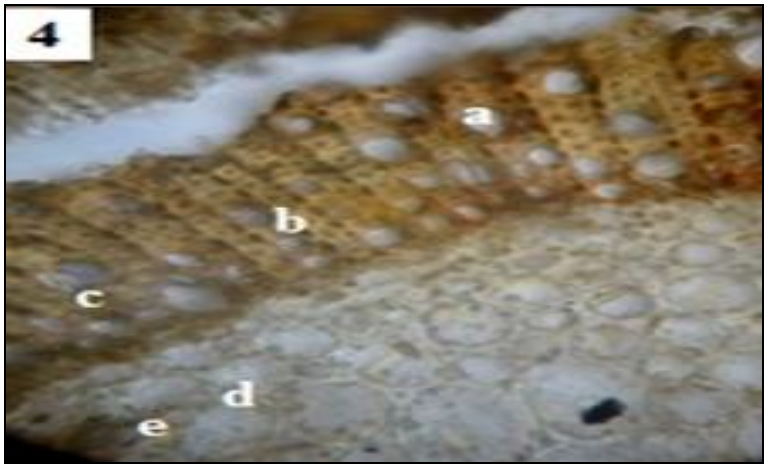

FIG. 4: REPRESENTATATIVE PHOTOMICROGRAPH OF TRANSVERSE SECTION OF STEM OF *J. MULTIFLORUM* AT 45 X SHOWING (A) VESSELS; (B) XYLEM CELLS; (C) MEDULLARY RAYS; (D) PARENCHYMA; (I) FIBRE BUNDLE; (E) CALCIUM OXALATE CRYSTAL

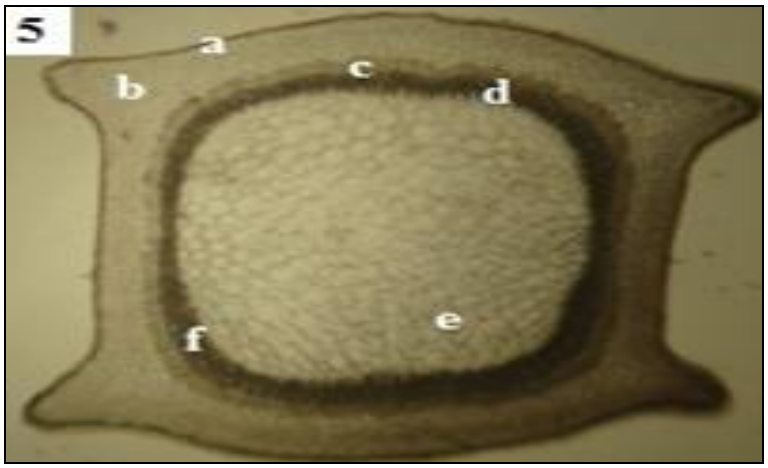

FIG. 5: MICROSCOPY OF TRANSVERSE SECTION OF *J. MESNYI* SHOWING AT 10X (A) EPIDERMIS; (B) COLLENCHYMAS; (C) ENDODERMIS; (D) MEDULLARY RAYS; (E) PARENCHYMA (F) XYLEM CELLS AND VESSELS

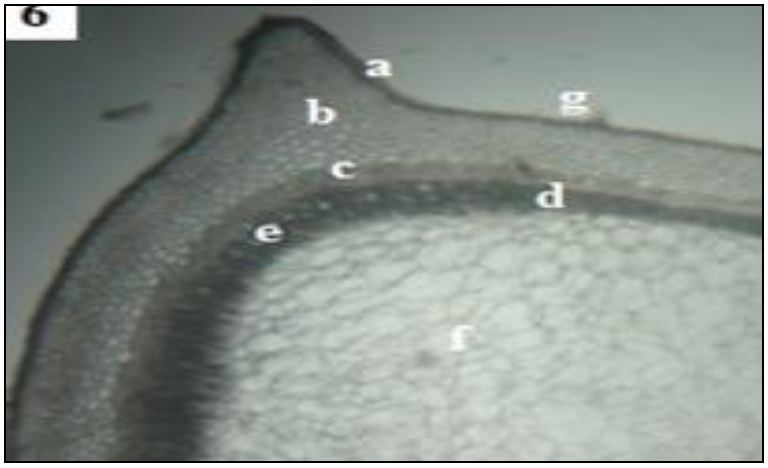

FIG. 6: MICROSCOPY OF TRANSVERSE SECTION OF *J. MESNYI* SHOWING AT 45X (A) EPIDERMIS; (B) COLLENCHYMAS; (C) ENDODERMIS; (D) MEDULLARY RAYS; (E) XYLEM CELLS AND VESSELS; (F) PARENCHYMA AND (G) TRICHOMES

Powder microscopic characters of the stem of *J. multiflorum* showed the presence of trichomes, parenchymatous cells and xylem fibre as shown in **Fig. 7** and *J. mesnyi* showed the presence of parenchymatous cells and xylem fibre as shown in **Fig. 8.**

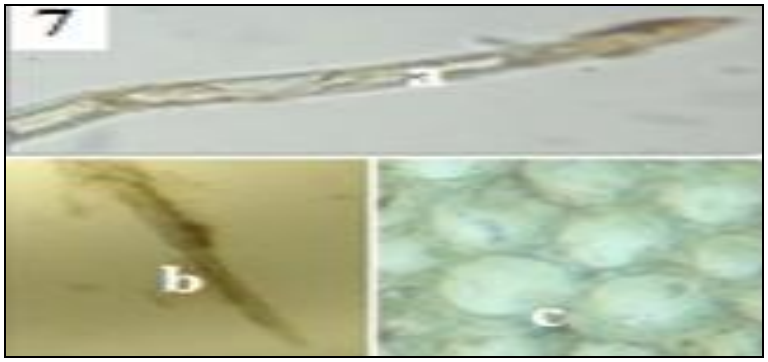

FIG. 7: POWDER MICROSCOPY OF STEM OF *J. MULTIFLORUMAT* 45X (A) TRICHOME; (B) XYLEM FIBRE AND (C) PARENCHYMATOUS CELL

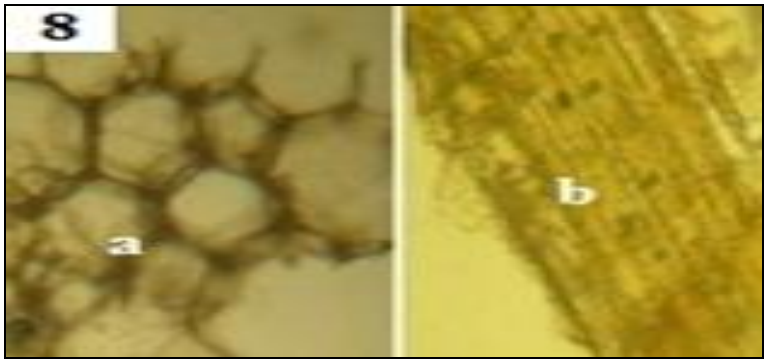

FIG. 8: POWDER MICROSCOPY OF STEM OF *J. MESNYI* AT 45X (A) PARENCHYMATOUS CELLS AND (B) XYLEM FIBRE

Fluoresence Study: The fluorescence behavior of the powder of both stems, moistened with different chemical reagents and solvents; under UV and daylight as shown in **Table 1**.

Physicochemical Parameters and Preliminary Phytochemical Screening: The ash values (total ash, water-soluble ash and acid-insoluble ash content), extractive values (ethanol-soluble and water-soluble extractive values), loss on drying, swelling index, foaming index and crude fibre content were determined in **Table 2**. The powder of stem was extracted successively using solvents of increasing polarity *i.e.* petroleum ether, chloroform, ethanol and water respectively.

TABLE 1: FLOURESCENCE STUDY OF STEM POWDER WITH DIFFERENT REAGENTS

Reagent	*J. multiflorum*			*J. mesnyi*		
	Day light	**Short UV (254nm)**	**Long UV (365nm)**	**Day light**	**Short UV (254nm)**	**Long UV (365nm)**
Dry drug	Dark green	Blackish	Green	Light green	Blackish green	Green
Drug + Distilled water	Green	Blackish	Blackish green	Green	Blackish	Blackish green
Drug +1N HCl	Dark green	Blackish	Blackish green	Dark green	Blackish grey	Dark green
Drug+ 50% HCl	Brownish green	Blackish	Blackish green	Green	Blackish	Blackish green
Drug + Acetic acid	Dark green	Blackish	Blackish green	Light green	Blackish	Brownish green
Drug + 50% H_2SO_4	Green	Blackish	Blackish green	Dark green	Smoky black	Blackish green
Drug +1N H_2SO_4	Brownish green	Blackish	Blackish green	Brownish green	Blackish	Brownish green
Drug +1N NaOH (Aq)	Brown	Blackish	Blackish green	Black green	Blackish	Brownish green
Drug +1N NaOH (Alc)	Black green	Blackish	Blackish green	Brown	Blackish	Brownish green
Drug+ $1NHNO_3$	Brown	Blackish	Dark Green	Brownish green	Blackish	Dark Green
$FeCl_3$ 5% (Alc)	Brownish green	Blackish green	Blackish green	Green	Blackish green	Blackish green
$FeCl_3$ 5% (Aq)	Brownish green	Blackish green	Blackish green	Green	Blackish green	Blackish green

Color and percentage yield of each were observed in **Table 3**. The Preliminary phytochemical screening depicted in **Table 4**, showed the presence of alkaloids, anthraquinone glycosides, saponins glycosides, cardiac glycosides, flavonoids, steroids, terpenoids, tannins and phenolic compounds. The Heavy metal content, alfatoxins and microbial content were observed within given limits as per WHO as shown in **Table 5**. The pesticide residues were observed according to WHO guidelines as given in **Table 6**.

TABLE 2: RESULT FOR PHYSICOCHEMICAL PARAMETERS

Parameters	*J. multiflorum*	*J. mesnyi*
Loss on drying	5.73%	6.54%
Total ash	3.92%	4.56%
Water soluble ash	1.75%	2.34%
Acid insoluble ash	1.28%	1.66%
Ethanol extractive	5.67%	8.81%
Water extractive	4.64%	7.16%
Swelling Index	Nil	Nil
Crude fiber content	7.83%	6.53%
Foaming Index	Less than 100	Less than 100

TABLE 3: RESULT FOR SUCCESSIVE EXTRACTS

Extract	*J. multiflorum*		*J. mesnyi*	
	Colour	Extractive value (%w/w)	Colour	Extractive value (%w/w)
Petroleum Ether	Blackish yellow	5.85	Yellowish brown	3.81
Choloroform	Yellowish green	6.74	Greenish black	4.56
Ethanol	Yellowish green	8.23	Dark brown	9.98
Aqueous	brownish	7.12	Dark brown	8.96

TABLE 4: RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING

Compounds	*J. multiflorum*				*J. mesnyi*			
	PE	CH	Et.	Aq.	PE	CH	Et.	Aq.
Alkaloids	-	-	++	++	-	-	++	-
Carbohydrates	-	-	++	++	-	-	++	++
Anthraquinone glycoside	-	-	-	-	-	-	-	-
Saponin glycoside	-	-	++	++	-	-	++	++
Cardiac glycoside	-	-	++	++	-	-	++	++
Coumarin glycoside	-	-	++	++	-	-	++	++
Flavanoids	-	-	++	++	-	-	++	++
Tannins & Phenolic	-	-	++	++	-	-	++	++
Protein and amino acids	-	-	++	++	-	-	++	++
Steroids & Terpenoids	++	++	++	-	++	++	++	-

Key: (++) = Present (-) = Absent # PE- Petroleum ether, CH- Chloroform, Et- Ethanol, Aq- Aqueous.

TABLE 5: PHYSICOCHEMICAL PARAMETERS

Parameter	Specified limit	Value	
		J. multiflorum	*J. mesnyi*
	Microbial Contamination Test		
Total Bacterial Count	1 X 10^5c.f.u./g	700	500
Total yeast and mould count	1 X 10^3 c.f.u/g	Nil	Nil
E. coli	Nil	Nil	Nil
Salmonella sp.	Nil	Nil	Nil
S. aureus	Nil	Nil	Nil
P. aeruginosa	Nil	Nil	Nil
	Aflatoxin Content		
Aflatoxin B1	0.5ppm	Nil	Nil
Aflatoxin B2	0.1ppm	Nil	Nil
Aflatoxin G1	0.5ppm	Nil	Nil
Aflatoxin G2	0.1ppm	Nil	Nil
	Heavy Metal Analysis		
Arsenic	5ppm	ND	0.01
Cadmium	0.3ppm	0.02	0.02
Lead	10ppm	0.01	0.01
Mercury	0.2ppm	ND	ND

ND- not detected.

TABLE 6: DETERMINATION OF PESTICIDES

Pesticides	Specified limit	Value	
		J. multiflorum	*J. mesnyi*
Alachlor	0.02	ND	ND
Atrazine	-	ND	ND
BHC (sum of all isomers)	0.3	ND	ND
Bifenthrin	-	ND	ND
Butachlor	-	ND	ND
Carbofuran	-	ND	ND
Carbofuran, 3-Hydroxy	-	ND	ND
Chlordane (sum of cis-, alpha-)	0.05	ND	ND
Cypermethrin peak 1	1.0	ND	ND
DDD(sum of all isomers)	1.0	ND	ND
DDE (sum of all isomers)	1.0	ND	ND
Dieldrin	0.05	ND	ND
Dimethoate	0.5	ND	ND
Edifenphos	-	ND	ND
Endosulfan peak 1	3.0	ND	ND
Endosulfan peak 2	3.0	ND	ND
Endosulfan sulphate	3.0	ND	ND
Endrin	0.05	ND	ND
Ethion	2.0	ND	ND
Fenthion	0.5	ND	ND
Fenvalerate	1.5	ND	ND
Heptachlor	0.05	ND	ND
Heptachlor epoxide	0.05	ND	ND
Malathion	1.0	ND	ND
Methoxychlor	-	ND	ND
Parathion-methyl	0.2	ND	ND
Phorate	-	ND	ND
Phoratesulfone	-	ND	ND
Phosalone	0.1	ND	ND

#ND- not detected.

DISCUSSION: Despite the availability of various analytical tools in the field of modern pharmaceuticals, still lacking pharmacognostical standards for traditionally used plants is the major lacuna. Precisely for the reason, the WHO has recommended various physicochemical methods for authentication of plant drugs. These parameters can even help in the detection of adulteration hencethey should be adopted to confirm the identity, purity, and quality of plant drugs [34].

Macroscopic parameters *viz.*, color, taste, texture, odour, size and shape are helpful for the identification of plant materials So, quality control of herbal drugs has traditionally been done based on appearance or morphological evaluation; but today microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. Physicochemical parameters are important parameters in detecting adulteration and are adopted to confirm the identity, purity, and quality of the drug. Ash values are particularly important parameters as these show the presence or absence of foreign matters like metallic salts and silica etc. The values of total ash, water-soluble ash, and acid-insoluble ash are 3.92%, 1.75% and 1.28% (*J. multiflorum*) and 4.56%, 2.34% and 1.66% (*J. mesnyi*). Loss on drying for both the stem was nearly 5.73% and 6.54% respectively.

It signifies the considerable amount of moisture in the stem. The moisture content of a drug should below in order to prevent decomposition of crude drug either due to chemical change of constituents or microbial contamination. The result of fluorescence analysis of stem powder showed their characteristic fluorescent color in different organic and inorganic solvents. The fluorescence behavior of powdered drugs plays a vital role in the determination of the identity, quality, and purity of the drug material.

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The result suggests that the drug has high ethanol-soluble extractive value compared to water-soluble extractive value. Preliminary Phytochemical Evaluation showed the presence of alkaloids, glycosides, saponins, steroids, terpenoids, flavonoids, carbohydrates, protein and amino acid, tannins and phenolic compounds in the stem of both plants. Heavy metal like mercury is not detected in any of the species however the level of cadmium, arsenic and lead are within the limits as per WHO guidelines. The results of microbial content, aflatoxin content, and pesticide residue revealed that both species are free from above the natural contaminants and therefore used for the preparation of formulation [34].

CONCLUSION: Standardization studies help in identification, authentication and establishing the quality of the plant material and will be useful in preparing a monograph of the plant. Further, it will act as a tool to detect adulterants and substituents and is helpful in maintaining the reproducibility of quality and efficacy of natural drugs.

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