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EVALUATION OF PHARMACOGNOSTICAL AND PHYSICOCHEMICAL PARAMETERS OF STEM OF *ACACIA ARABICA* WILLD.

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Acacia arabica, *Acacia nilotica*, Microscopy, Physicochemical, Standardization

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ABSTRACT: *Acacia Arabica* Willd.(syn: *Acacia nilotica)* is a thorny tree belonging to the family Fabaceae and subfamily Mimosoideae. Gum acacia, an exudation obtained from this plant, is included in the GRAS category and used widely as pharmaceutical aid. The plant contains flavonoids, tannins, and phenolic compounds as its phytoconstituents. The plant possesses antioxidant, antipyretic, antimicrobial, antiviral, antifungal, spasmolytic, antibacterial, hypoglycaemic, antifertility, immune-modulatory, hepatoprotective, hypotensive, and wound healing properties. The present study was done for the assessment of macroscopic characters, microscopic characters and physicochemical parameters. The chemical evaluation was done to identify the identification of active constituents of the plant. Other standardization methods like ash values, extractive values, and moisture content were estimated as per the standard process prescribed in IP. Heavy metal, aflatoxin, microbial contamination, and pesticide residue have also been calculated per the WHO guiding principle. *A. arabica* is brownish red, cylindrical with fibrous texture, hard to break, and rough to touch. Microscopic characters showed the presence of epidermis, collenchyma, vessels, medullary rays, xylem cells, calcium oxalate crystals, and pith. Heavy metals, aflatoxins, pesticide residues, and microbial count were found within limits per WHO guidelines. Phytochemical screening showed the presence of tannins, phenolic compounds, and flavonoids. The present study was carried out to establish quality parameters for *A. arabica*, which will be further utilized for the evaluation of pharmacological activity.

INTRODUCTION: *Acacia Arabica* (Synonym: *Acacia nilotica* Willd. Ex. Del., *Mimosa arabica*, *Mimosa nilotica*), commonly known as babul or keekar is a tree that grows up to 20 m in height with a dense spheric crown; belonging to the subfamily Mimosoideae of the family Fabaceae.

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A.arabica is widely distributed throughout the drier parts of India, Ceylon, Baluchistan, Waziristan, Arabia, Egypt, tropical Africa and Middle Asia [1].

The plant is rich in tannins and polyphenolic compounds such as catechin, epicatechin, (+)dicatechin, epigallo catechin, gallic acid & its methyl ester, quercetin, catechol, epicatechol, (+)-leucocyanidine gallate, kaempferol-3-glucoside, isoquercetin, leucocyanidin, hentriacontane, hentriacontanol, betulin, sitosterol, polysaccharides *viz*. 6-O-(β-glucopyranosyluronic acid)-D-galactose, 6- O-(4-O - methyl – β – D - glucopyranosyluronic acid)-Dgalactose, 4-O-(α-D-

glucopyranosyluronic acid)-Dgalactose and 4-O-(4-O-methyl-α-D-gluco-pyranosyluronic acid)-D-galactose. It also contains 3, 5-di-O-methyl-L-arabinos, 2-O-β-Larabino-pyranosyl-L-arabinose, arabinobiose, 3-O-β-Larabinopyranosyl-L-arabinose [2-6]. Plant is utilized for antifertility [7], hypotensive, spasmolytic, hypoglycaemic, CNS depressant, anti-fungal, aphrodisiac, haemostatic, antipyretic properties [2]. The plant has already been screened for various biological activities such as anti-oxidant [6, 8], chemopreventive [9, 10], anthelmintic [11], antimutagenic [10], cytotoxic [12], antimicrobial [13, 14], anti-inflammatory [15] and sexually transmitted infections [16]. Many developing countries have relied upon natural products for managing various diseases since long ago, making it an area of interest for research. Pharmacognostical standardization is a critical footstep in search of a better lead product from natural sources. The current study was considered to evaluate various standardization parameters to safeguard the use of the plant for further studies.

MATERIALS AND METHODS:

Plant material and Extract Preparation: *A. arabica* stems were collected from the surroundings of Sonepat, Haryana. The plant was identified by Dr. Sunita Garg (Emeritus Scientist, CSIR-NISCAIR, New Delhi) under a voucher specimen number-NISCAIR/RHMD/ Consult /2018/3295-96-2 (Nov. 28, 2018). A copy of the same was submitted to the Department of Pharmacognosy, Hindu College of Pharmacy, Sonepat, Haryana, for further reference (AN-01). The stem was shredded into small pieces and dried at room temperature. The dried stem was coarsely powdered and stored in an airtight container for further studies. The plant material was successfully extracted by soxhlation process using polar and non-polar solvents such as petroleum ether (60-80°C), chloroform and ethanol (95%). The decoction technique for extraction was employed for the preparation of aqueous Extract. Extracts were concentrated under a rota-evaporator and stored in a desiccator for further use. Reagents and chemicals were purchased from RFCL, Mumbai, India.

Macroscopic Characters: The drug's colour, shape, and size were observed under daylight. The texture and odour of the plant were noticed by touching and rubbing the sample drug, respectively [17, 18].

Microscopic Studies: The thin transverse sections of the fresh stem were cut by free hand using a sharp blade. Fine sections were mounted with different stains, *viz.* phloroglucinol, hydrochloric acid, ruthenium red, safranin, and glycerine. Fine powder (passed through #60 sieve) was used for powder microscopy using the same process stated above. Microscopic photographs were captured using a Sony camera attached to an ATC-2000 microscope [17-20].

Physicochemical Studies: Extractive values, swelling index, moisture content, total ash content, acid-insoluble ash value, and water-soluble ash value were determined as per the procedure given in IP [17-20]. Preliminary phytochemical screening was done to identify the presence of various phytoconstituents using standard methods [17-22]. The drug was processed to observe fluorescence behaviour as per standard procedure [23]. Aflatoxin content, heavy metal analysis, pesticide residual values and microbial contamination were evaluated for the plant per WHO guidelines [20, 22, 24].

RESULTS AND DISCUSSION:

Morphological Studies: The longitudinally cut portion of the stem showed brownish red heartwood encircled by brownish white sapwood. The size and colour of the trunk change with the growth of the plant. The stem was tubular with a rough texture and a touch, as shown in **Fig. 1**.

FIG. 1: PHOTOGRAPH OF *A. ARABICA* PLANT SHOWING A) FRESH LEAVES, FLOWER AND BRANCHES; B) INTERNAL SURFACE OF PLANT STEM SHOWING HEARTWOOD AND SAPWOOD; C) EXTERNAL SURFACE OF PLANT STEM SHOWING BARK

The fracture was hard and fibrous. The outer surface of the plant stem bark showed a brownish

grey colour, while the inner surface was brownish red. The stem powder was dark brown in colour without any odour or taste.

Microscopical Studies: The transverse section of a fresh stem presented a round-shaped image with curvy epidermis. A thin transverse section showed the presence of collenchyma, medullary rays, parenchyma, vessels, xylem cells and pith. Medullary rays are uni to multi- seriate and run almost straight; vessels exist as isolated or in groups of two to four. Calcium oxalate crystals are found scattered amongst the secondary cortex and phloem parenchyma stone cells. A few cells are filled with yellowish brown coloured fluid, as shown in **Fig. 2-4**. Under the microscope, the reddish-brown coloured powder showed the presence of lignified cells, cork cells, xylem fibers, sclereids, pitted cells and fibres, as shown in **Fig. 5**.

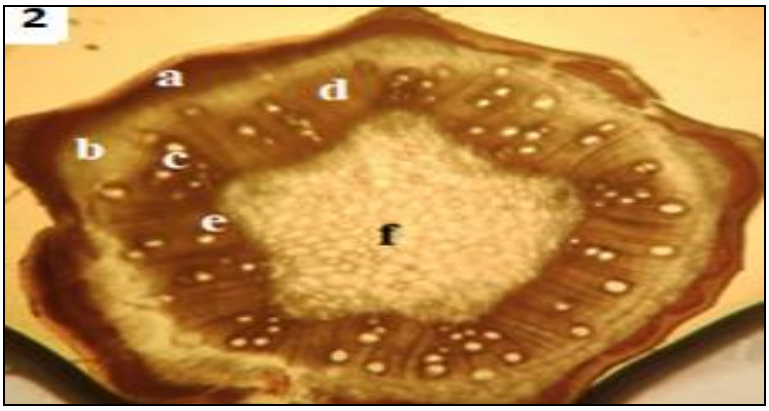

FIG. 2: PICTOMICROGRAPH OF *A. ARABICA* STEM AT 10X SHOWING A) EPIDERMIS; B) COLLENCHYMA; C) VESSELS; D) MEDULLARY RAYS; E) XYLEM CELLS AND F) PITH

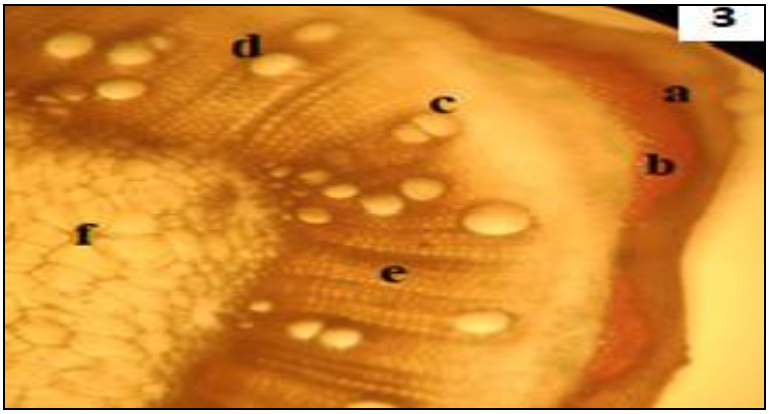

FIG. 3: PICTOMICROGRAPH OF T.S. *A. ARABICA* SHOWING A) EPIDERMIS B) PHLOEM FILLED WITH YELLOW COLOURED LIQUID; C) XYLEM CELLS; D) VESSEL; E) MEDULLARY RAYS AND F) PITH AT 45X

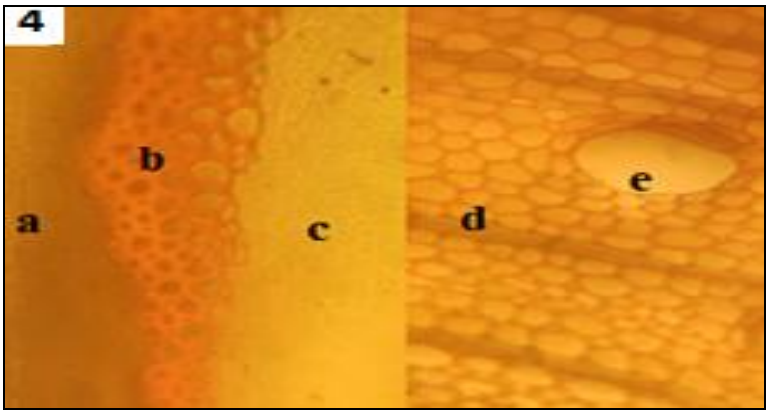

FIG. 4: MICROPHOTOGRAPH OF T.S OF *A. ARABICA* AT 45X SHOWING A) CORTEX; B) CELLS CONTAINING CALCIUM OXALATE CRYSTALS AND STONE CELLS; C) COLLENCHYMA; D) MEDULLARY RAYS AND E) VESSEL

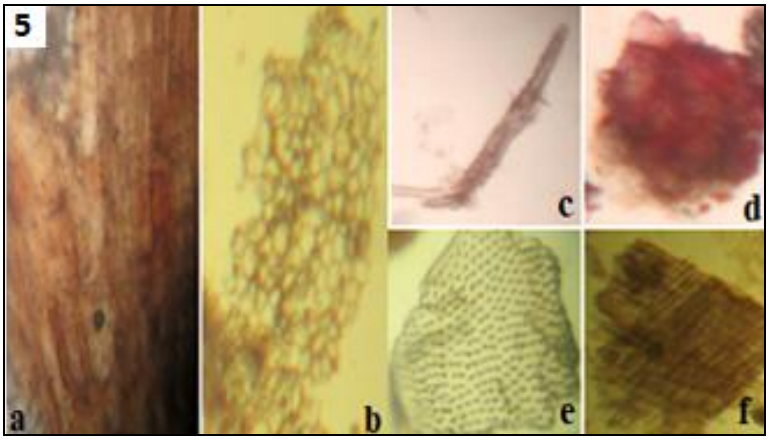

FIG. 5: MICROPHOTOGRAPH OF *A. ARABICA* AT 45X SHOWING A) LIGNIFIED CELLS; (B) CORK CELLS; (C)XYLEM FIBRE; (D) SCLEREIDS; E) PITTED CELLS AND F) FIBRE

Physicochemical Parameters: Total ash content, water-soluble and acid-insoluble ash content, water, and ethanol-soluble extractive values, loss on drying and swelling index were assessed as per standard procedure given in IP, and observations are stated in **Table 1**.

Successive extracts were obtained by soxhlation using solvents such as petroleum ether, chloroform, ethanol, and water, respectively. The appearance and percentage values of successive extracts are given in **Table 2**.

Phytochemical screening of stems revealed the presence of flavonoids, tannins, phenolic compounds, saponins, carbohydrates and lipids as indicated in **Table 3**. **Table 4** summarises observations on drug fluorescence behavior. Values for heavy metal content, microbial content, aflatoxins and pesticide residues were evaluated according to WHO guidelines, and the results are shown in **Table 5**.

TABLE 1: RESULT FOR PHYSICOCHEMICAL PARAMETERS

S. no.	Parameter	*A. arabica*
1	Total Ash Content	7.8% w/w
2	Acid-insoluble ash value	1.5% w/w
3	Water soluble ash value	1.6% w/w
4	Loss on drying	10.5%w/w
5	Alcohol soluble extractive value	6.4% w/w
6	Water soluble extractive value	4.6% w/w
7	Swelling Index	NIL

TABLE 2: RESULT FOR SUCCESSIVE EXTRACTS

S. no.	Extract	Colour	%age Extractive Value
1	Pet. Ether	Brown	0.15%
2	Chloroform	Brown	0.95%
3	Ethanolic	Dark brown	5.59%
4	Aqueous	Brownish black	5.58%

TABLE 3: PHYTOCHEMICAL SCREENING OF *A. ARABICA* STEM

S. no.	Compounds	PE	CH	Et.	Aq.
1	Alkaloids	--	--	--	--
2	Carbohydrates	--	--	--	--
3	Steroids	--	--	--	--
4	Saponins	--	--	*+ve*	*+ve*
5	Proteins	--	--	--	--
6	Fixed Oils/ Fats	*+ve*	--	--	--
7	Flavanoids	--	--	*+ve*	*+ve*
8	Tannins & Phenols	--	--	*+ve*	*+ve*
9	Gums&Mucilages	--	--	--	--
10	Glycosides	--	--	--	--

PE- Petroleum ether extract; CH-Chloroform Extract; Et- Ethanol extract; Aq- Aqueous Extract.

TABLE 4: FLUORESCENCE BEHAVIOR OF *A. ARABICA* WITH DIFFERENT REAGENTS

S. no.	Reagent	Colour in ordinary light	Colour under UV light	
			Short(254nm)	Long (365nm)
1.	Powder as such	Reddish Brown	Light Brown	Brown
2.	1N NaOH in Methanol	Yellowish Brown	Dark Green	Violet

3.	1N NaOH in Water	Reddish Brown	Brown	Blackish Brown
4.	1N HCl	Dark Brown	Brown	Violet
5.	50% HNO_3	Yellowish Brown	Green	Dark Violet
6.	50% HCl	Reddish Brown	Dark Green	Black
7.	50% H_2SO_4	Dark Brown	Brownish Green	Dark Violet

TABLE 5: PHYSICOCHEMICAL PARAMETERS

Parameter	*A. arabica* value	Specified limit
Microbial contamination test		
Total Bacterial Count	960	1 X 10^5 c.f.u./g
Total yeast & mould count	Nil	1 X 10^3 c.f.u/g
E. coli	Nil	Nil
Salmonella sp.	Nil	Nil
S. aureus	Nil	Nil
P. aeruginosa	Nil	Nil
Aflatoxin content		
Aflatoxin B1	Nil	0.5
Aflatoxin B2	Nil	0.1
Aflatoxin G1	Nil	0.5
Aflatoxin G2	Nil	0.1
Heavy metal analysis		
Arsenic	ND	5
Cadmium	ND	0.3
Lead	0.04	10
Mercury	ND	0.2
Pesticides		
Alachlor	ND	0.02
Atrazine	ND	-
BHC (sum of all isomers)	ND	0.3
Bifenthrin	ND	-
Butachlor	ND	-
Carbofuran	ND	-
Carbofuran, 3-Hydroxy	ND	-
Chlordane (sum of cis-, alpha-)	ND	0.05
Cypermethrin peak 1	ND	1.0
DDD(sum of all isomers)	ND	1.0
DDE (sum of all isomers)	ND	1.0
Dieldrin	ND	0.05
Dimethoate	ND	0.5
Edifenphos	ND	-
Endosulfan peak 1	ND	3.0
Endosulfan peak 2	ND	3.0
Endosulfan sulphate	ND	3.0
Endrin	ND	0.05
Ethion	ND	2.0
Fenthion	ND	0.5
Fenvalerate	ND	1.5
Heptachlor	ND	0.05
Heptachlor epoxide	ND	0.05
Malathion	ND	1.0
Methoxychlor	ND	-
Parathion-methyl	ND	0.2
Phorate	ND	-
Phoratesulfone	ND	-
Phosalone	ND	0.1

#ND= not detected

DISCUSSION: In this modern era of science and technology, research is booming with the help of various analytical tools. As the use of herbal drugs is increasing in the medicinal industry, herbal drug standardization has become an essential part of the research process. WHO has already recommended

many physicochemical processes for the standardization of plants. These procedures are beneficial in omitting any kind of adulteration from the raw material and ensure the drug's safety, efficacy, and acceptability [25-27]. Macroscopic or morphological parameters have traditionally been used to identify drugs using sense organs. Still, for requisite identification, microscopic evaluation plays a vital role in uncovering the presence of foreign matter or any admixing. Preliminary studies and physicochemical standards deliver important information about the drug, which is helpful in routine industrial production. Loss on the drying test indicates the water and volatile matter present in the drug. Ash value is obtained after igniting the specific quantity of material; acid insoluble ash value points toward foreign matter like metallic salts and silica. Extractive value signifies the uniformity of nature and quantity of chemical constituents present in the drug.

Fluorescence studies of powdered drugs with various chemicals give an idea about the chemical nature of the crude drug. Heavy metals and pesticides are dangerous toxic trace elements, as stated by WHO. Aflatoxins are toxic and carcinogenic secondary metabolites produced by fungal species. Underprivileged hygienic conditions may lead to microbial contamination of raw material, which may lead to harmful chemical degradation of constituents. It is mandatory to control the contamination of medicinal plants with heavy metals, microbial content, aflatoxins, and pesticide residue to safeguard human health. In view of the importance of pharmacognostic and physicochemical parameters, *A. arabica* was characterized by evaluating organoleptic and microscopic characters. The plant was assessed for extractive values, ash content, moisture content, fluorescence nature, phytochemicals, heavy metals, aflatoxins, microbial content and pesticide residues.

CONCLUSION: Any plant's macroscopic, microscopic and chemical evaluation is a necessary step toward standardization. These variables indicate any adulteration or substitution in the drug and ensure the quality and purity of the plant. In this regard, *A. arabica* stem was subjected to the standardization process. Chemical identification indicated the presence of flavonoids, phenolic compounds, and tannins. All physicochemical parameters were found within limits during the research work. In this respect, the plant has been authenticated for all standard parameters before proceeding toward pharmacological studies.

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CONFLICTS OF INTEREST: The article has no conflict of interest.

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