Pharamacognostic Profile and Comparative *In Vitro* Anti-Inflammatory Activity Study of Ethanomedicinal Plants of North East India

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ABSTRACT

Background: In ethno-medicinal practices, the leaves of *Alocasia macrrorhizos, Canavalia ensiformis, Amaranthus cruentus, Averrhoea carambola, Bauhina tenifloara,* and *Capsicum Chinese* have different pharmacological activities. The problem encountered with the proper utilization of herbal drugs is lack of documentation and standard parameter, which create problem in acceptance and importance of these plants. **Objective:** The objectives of our study are to establish scientifically evaluated pharmacognostic profile and *in vitro* anti-inflammatory screening of six plants. **Methods:** Leaf sample from each plant was evaluated by macroscopic, microscopic and physicochemical parameters (As per WHO recommended methods of standardization). The anti-inflammatory activity screening of methanolic extracts was carried out against inhibition of protein denaturation method taking diclofenac sodium as a benchmark. **Result:** The macroscopic characteristic and microscopical evaluation reveals the type of stomata within the leaves and presence of parenchyma cells, collenchyma cells, upper epidemis, lower epidermis, vascular bundle, palisade cells and trichomes in the transverse section of leaves. Physicochemical parameter reveals the total ash, acid insoluble ash, water soluble, methanol soluble extractives and moisture content. The IC_{50} value of MEAM, MECE, MEAC, MEAC1, MECC, and MEBT against inhibiton of protein denaturation was found to be 72.88, 841.78, 735.12, 303.75, 188, and 354.1855 µg/mL respectively. **Conclusion:** The present study contributes useful information that will help in the exact identification as well as assessment of purity of crude drugs. *Methanolic extract of Alocasia macrorrhizos could be a potential anti-inflammatory agent from the natural sources.*

Key words: Pharmacognostic study, Anti-inflammatory, Microscopic, Macroscopic, Diclofenac sodium, Northeast India.

INTRODUCTION

Herbal drugs play a vital role in health care programmes especially in developing countries. However there are hindrances after the adoption of alternative medicines in developed countries as there is the lack of documentation and good quality testing of crude drug. Proper documentation and practice of herbal remedies are essential to the universal acceptance of this medicine. With the inclusion of Pharmacognostic standardization, physico-chemical parameter studies are worldwide accepted for the identification and validation of plant material. Proper identification and quality assurance of plant material is required to ensure the productive effects of herbal medicines, which will provide safety and effectiveness. Pharmacognostic formulation of plant material includes its behavioral, structural and chemical properties.[1] *Averrhoa carambola* is an ethanomedicinal plant of the family Oxalidaceae, a decoction of the leaves mixed with mastic (rock sugar) taken orally in the treatment of kidney stone by the people of Manipuri.[2] It is best known as "Star Fruit" or "Kamrakh". It has various medicinal properties such as antipruitic, antipyretic, anthelmintic, anti-inflammatory, anti-ulcer and antimicrobial. It has an adequate presence

of L-ascorbic acid, (-) epicatechin and gallic acid because it could be used as a natural antioxidant.[3] It has been reported that the anti-inflammatory effects of topical extracts produced by raw ethanolic extracts of *Averrhoa carambola* in skin inflammation.[4] *Canvalia ensiformis* belongs to the papilionaceae family and is native to a relatively small region in the North East region.[5] This plant has some properties, canavine is a major toxin, present in 2-3.5% of dried seeds, and inhibits protein metabolism.[6] In traditional medicine Sword bean is used for hiccups, vomiting, abdominal swelling, lumbago (lower back pain) due to kidney deficiency, asthma with sputum.[7] *Allocasia macrorrhiza* belongs to the family araceae, the use of plants of the people of the Zeliang tribe of Nagaland, about 10ml of fluid released after cutting into the rhizome and leaves is inserted into a snake bite with cow's milk or buffalo immediately after being bitten to remove the venom.[7] *Allocasia macrorrhiza* rhizome paste -1g is added as a *capsicum annum* infused into a ripe banana once daily for four days in hepato-gastric diseases. This recipe is for adults only and is a common remedy among santhals, a tea garden tribe of Assam.[8] *Capsicum chinese* (Naga King Chili) is an important spice crop of India belongs to solanaceae family. It is a very pungent chili, measuring

10, 01,304 Scoville Heat Units (SHU). It is locally called the Naga King Chili (Bhoot jolokia or Naga jolokia in Assamese), and originates in the northeastern provinces of India especially Nagaland.[9] It is traditionally used as good appetizer, asthama, and for curing of heart diseases by Zeliang tribe Nagaland.[7] *Amranthus viridis* belongs to the amranthaceae family, leaves of these plant is traditionally used to relief from diarrhea, gastroenteritis, fever, haemorrhage, anaemia or kidney complaints due to the presence of different phytochemicals.[10,11] It also has diuretic properties. The roots of the plants are boiled with honey and used for laxative properties in infants. Ash from stems is used as a wound dressing and hot leaves are used for tumor diseases.[12] *Bauhinia teniflora* is belonging to the caesalpiniaceae, the bark decoction is taken in leprosy and dysentery and its infusion in venom bites.[13] Inflammation is the normal physiological response of the immune system to tissue injury caused by various physical trauma, toxic chemical or microbial agents. It is the body's response to neutralizing or destroying invasive organisms to contribute to neutralizing and regenerating body tissue. It is activated by the release of chemical mediators from injured tissues and migrating cells.[14] Drugs used for the management of inflammatory condition are non-steroidal anti-inflammatory drugs, which have lots of adverse effects especially gastric irritation leading to formation of gastric.[15] Natural organic compounds can be used as marker compounds in either synthesis or semi-synthesis processes so that they play an important role in the development of new drugs based on therapeutic targets. Misidentification of natural products or herbal medicines is also the reason for its use, [16] For example, the existence of vernacular names given to two or more completely different species. All these problems can be solved by pharmacognostic studies in plants. It is therefore the present study that describes comparative *in vitro* anti-inflammatory screening of methanolic extracts against protein denaturation, as well as pharmacological standardization through macroscopic, microscopic and physiochemical analysis.

MATERIALS AND METHODS

Plant materials, collection and identification

Fresh plants materials were collected from different parts of North east India. The plants specimens were authenticated by Dr. A. A. Mao (Scientist-F & H) Botanical Survey of India, Shillong. A voucher specimens of these plants have been deposited in the Department of Pharmacy, with Reference no: BSI/ERC/TECH/2017/589 for future reference.

Plant material extraction

After the collection of plant material, the fresh leaves were spread and washed and dried for a week under shade. The dried leaves were finely ground into powder. The coarsely powdered leaves of the plant were then extracted completely by cold maceration. Six plants (*Allocasia macrorrhiza*, *Canavalia ensiformis*, *Amaranthus cruentus*, *Capsicum Chinese*, *Averrhoa carambola* and *Bauhinia teniflora*) were subjected to cold maceration technique for extraction. The powdered leaves of each plant were put in methanol at room temperature. Seven days later, the extracts were filtered through Whaman filter paper No. 1 under vacuum. The residue was again immersed in methanol for seven days and then filtered. The filtrate was combined and methanol was evaporated under vacuum, using rotary evaporator (Buchi Rotavpre R-200) at 45 ° C. Methanolic extracts were used for the assessment of anti-inflammatory activity studies.[17]

Chemicals and requirements

Phloroglucinol, chloral hydrate, glycerol, hydrochloric acid, methanol, ethanol, bovine serum albumin, diclofenac sodium, butylated hydroxyl anisole, and all other chemicals used in the study were of analytical grade obtained from Quali-Tech Laboratory Reagent, Himedia,

Loba Chemie Pvt. Ltd., Merck Pvt. Ltd. and SD Fine-Chem Limited. Projection microscope (ZEISS- True Chrome II), Rotary vacuum evaporator (IKA), electronic balance (Wenser), muffle furnace (Weiber-Thermotech), glasswares (Fisherbrand and Borosil), BOD Incubator (Thermolab), Laminar Air Flow, UV- Visible Spectrophotometer (Thermo Fisher Scientific), etc. were utilised for the study.

Pharamcognostic evaluation

Macroscopic

Macroscopic characterization ensures that crude drugs can distinguish them between closely related species. Macroscopy of a drug involves its visual appearance to the naked eye. It also depends to a large extent on the part of the plant from where the medicine is obtained. For each particular morphological group, a special systemic test can be performed. Size, color, smell and taste are significant parts of the morphology of a particular drug.[18,19]

Transverse section of leaf

The microscopic evaluation was done by cutting a transverse section of fresh leaves through the lamina and midrib, which was then treated with a phloroglucinol hydrochloride solution, it was mounted with glycerin, covered with cover slits, and observed under the microscope at 10X and then at 40X. The presence of followings was observed: epidermal cells (upper and lower), xylem, phloem, stomatous (anomocytic), collenchyma cells, and parenchyma cells.

Determination of stomatal number and stomatal index

The leaf piece was boiled with chloral hydrate solution. The upper and lower epidermis was peeled separately through forceps. It was placed on a slide and glycerin mounted in water and viewed under a projection microscope at 40X. The epidermal cell and stomata were counted in the region of 200 μm. The cell was included if at least half of its area is within the square. The result was recorded for each of the four regions and was calculated per sq.mm[16].

The stomatal index was calculated by using the formula: $I = S \times 100/E+S$

Determination of vein-islet and vein-termination number

The leaf of the plant, excluding the midrib, was cut into small pieces and boiled on a water bath with chloral hydrate solution in a 50 ml beaker. Then, the preparation was mounted on a glass slide with glycerin. The projection microscope was used for observation and evaluation of vein islet and vein termination at 10X. A square of 400μm was taken for the four regions and the number of vein islets and vein endings were counted for each section. Observations were taken as the range and the mean value indicated.[20]

Physicochemical evaluation

The determination of various physiochemical constants such as ash value, extractive value, and loss on drying were determined according to World Health Organization (WHO) guidelines. All evaluations were done in triplicate and expressed as mean values.[21-23]

In vitro- anti-inflammatory activity study inhibition of protein denaturation method

The test solution (0.5 ml) contains 0.45 ml (5% w / v aqueous solution) of BSA and the test extract solution (10, 20, 40, 60, 80, 100 μg / mL) of 0.05 ml. The test control solution (0.5 mL) contains 0.45 mL of BSA (5% w / v aqueous solution) and 0.05 mL methanol. The standard solution (0.5 mL) contained 0.45 mL (5% w / v aqueous solution) of BSA and 0.05 mL (10, 20, 40, 60, 80,100μg / mL) of diclofenac sodium. All the

Table 1: Solvent used in the extraction and % yield.

Sr. No.	Name of the Plant	Weight of drug taken	Solvent	Volume of solvent taken	% yield
1	*Alocasia macrorrhizos*	200gm		1 lit.	3.1
2	*Canavalia ensiformis*	200gm		1 lit.	3.9
3	*Amaranthus cruentus*	200gm	Methanol	1 lit.	3.6
4	*Averrhoa carambola*	200gm		1 lit.	4.5
5	*Bauhinia teniflora*	200gm		1 lit.	4.1
6	*Capsicum Chinese*	200gm		1litre	4.8

Table 2: Macroscopic characteristics of crude drugs.

Sr. No.	Parameter	Value					
		Alocasia macrorrhizos	*Canavaliaensiformis*	*Averrhoa carambola*	*Amaranthus cruentus*	*Capsicum Chinese*	*Bauhinia teniflora*
1	Size	Perennial, 3-6 ft	2-3 ft long, 4-5 inch wide	15- 20 cm	11-14 cm	7-11cm	7 – 9 cm
2	Colour	Green	Green	Greenish	Green to brown	Green	Green
3	Odour	Odourless	odourless	Aromatic	Spicy flavor	Mild flavor	Mild sweet scent
4	Taste	Acrid	Characteristic	Sour to mildy sweet	Spinach like	Sweet	Characteristics
5	Shape	Heart shape	Ovate	Oblong	Flabellate	Sagittate	Bilobed
6	Margin	Lobate	Undulate	Glabrous	Serrate	Magnetia	Glabrous
7	Apex	Acute	Rounded	Acute	Accuminate	Caudate	Obcordate
8	Base	Arrow	Acute	Oblique	Cordate	Cunate	Cordate

Table 3: Quantative study of leaf microscopy for the stomatal number, stomatal index, vein islet and vein termination number.

Sr. No.	Name of the Plant	Stomatal No. (Lower epidermis)	Stomatal No. (Upper epidermis)	Stomatal Index (Lower epidermis)	Stomatal Index (Upper epidermis)	Vein islet	Vein termination
1	*Allocasia macrorrhiza*	5	3	20.8	13.6	2	4
2	*Canavalia ensiformis*	3	1	18	8	2	3
3	*Averrhoa carambola*	6	4	14.6	13.2	3	6
4	*Amaranthus cruentus*	4	3	15.3	12.7	2	3
5	*Capsicum Chinese*	5	4	22.72	19.4	2	3
6	*Bauhinia teniflora*	4	3	33.3	26.2	6	5

Values are at 40X within 200 μm sq., where X-magnification power.

Table 4: Physicochemical parameter of crude drugs.

Sl No.	Name of the Plant	Total ash value	Acid insoluble ash	Water soluble ash	Moisture content	Alcohol soluble extractive
1	*Alocasia macrorrhizos*	16.3 ± 0.04	18.7 ± 0.05	6.2 ± 0.03	9.2 ± 0.04	11.2 ± 0.02
2	*Canavalia ensiformis*	13.4 ± 0.05	6.7 ± 0.08	8.9 ± 0.06	9.8 ± 0.01	6.8 ± 0.04
3	*Amaranthus cruentus*	15.4 ± 0.07	18.2 ± 0.03	11.9 ± 0.02	15.2 ± 0.06	9.1 ± 0.09
4	*Avarrhoea carambola*	19.2 ± 0.06	13.2 ± 0.02	15.7 ± 0.01	19.4 ± 0.07	11.5 ± 0.1
5	*Bauhinia teniflora*	9.5 ± 0.08	8.8 ± 0.05	4.2 ± 0.07	13.2 ± 0.03	5.9 ± 0.06
6	*Capsicum Chinese*	17.3 ± 0.03	12.7 ± 0.04	9.7 ± 0.05	9.2 ± 0.05	16.1 ± 0.08

Values are in (%w/w)

Table 5: Inhibition of protein denaturation of methanolic extracts of crude drugs.

S. No.	Dose (μg/mL)	% Inhibition (Mean ± SEM)						
		Diclofenac sodium	MEAH	MECE	MEAC	MEAC1	MECC	MEBT
1	10	43.9 ± 0.24	22.15 ± 0.55	4.62 ± 0.29	19.18 ± 0.26	25.98 ± 0.45	20.87 ± 0.66	30.53 ± 0.54
2	20	47.5 ± 0.26	25.74 ± 0.72	5.87 ± 0.41	19.29 ± 0.58	23.01 ± 0.32	18.53 ± 0.41	29.81 ± 0.54
3	40	56.3 ± 0.13	35.33 ± .0.27	4.94 ± 0.45	23.60 ± 0.36	32.17 ± 0.10	23.22 ± 0.32	35.67 ± 0.36
4	60	62.6 ± 0.17	52.18 ± 0.26	6.23 ± 0.28	23.47 ± 0.26	34.98 ± 0.33	34.57 ± 0.40	32.63 ± 0.63
5	80	65.2 ± 0.15	53.53 ± 0.28	6.64 ± 0.42	25.05 ± 0.57	33.22 ± 0.43	30.56 ± 0.29	38.26 ± 0.81
6	100	71.2 ± 0.18	57.46 ± 0.32	11.00 ± 0.58	21.31 ± 0.85	29.90 ± 0.20	33.30 ± 0.40	33.34 ± 0.60

Values are presented as Mean ± SEM, n=3

above solutions were adjusted to pH 6.3 using 1 N hydrochloric acid. The samples were incubated at 37 ° C for 20 minutes and amplified to keep the samples at 57 ° C for 3 minutes. After cooling, 2.5 mL of phosphate buffer was added to the above solutions. Absorbance was measured using a UV Visible Spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control-Absorbance of extracts}) \times 100}{\text{Absorbance of control}}$$

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium (10, 20, 40, 60, 80, 100µg/mL).[24]

DISCUSSION

Identification is an inevitable part of the standardization of crude drugs.[25] before the crude drugs are put in Herbal Pharmacopeia, the pharmacognostic profile and quality of raw drug samples must be specified. Macroscopy standards, microscopy, and quality of Simplicia can be used as a vital parameter in identification of crude drug.[26] The Macroscopic characteristics enables us to know the source of diagnostic parameters[27], and the microscopic method is one of the simplest and inexpensive methods, which helps us to identify plant samples.

Standardization becomes a crucial measure to determine the quality, quantity, purity, and authenticity of plants.[27] similarly; macroscopic observations of leaf characters such as size, color, odor, taste, shape, margin, apex, base and so on can help the identification of plants. Microscopy of leaves shows two subsidiary cells parallel to stoma. Hence it is paracytic type of stomata as seen in *Alocasia macrorrhizos*, *Averrhoa carambola*, *Capsicum chinese* while microscopy of the leaves shows the presence of anomocytic stomata as seen in *Canavalia ensiformis*, *Amaranthus cruentus*, *Bauhinia teniflora*. Microscopic evaluation can be noticed from the transverse section of the leaves, it includes trichomes, collenchyma, parenchyma, upper epidermis, lower epidermis, vascular bundles and palisade cells. The evaluation of physicochemical parameters helps in establishing pharmacopoeial standards. Ash value is one of the common parameters used to determine the identity and purity of a drug.[28] the values of total ash, acid insoluble ash and water soluble as depicted in Table 3. The Ash determines the purity of the crude drug in relation to the polarity of the solvent used and also help us to identify the nature of chemicals present in them.[29] Moisture content is related to the stability and quality of crude drugs because if the amount of water is high then the drug will deteriorate causing the biomass and its active principles to deteriorate.[30] The moisture content found on different plants were in the range of 9.2±0.04 to19.4±0.07. Leaves were
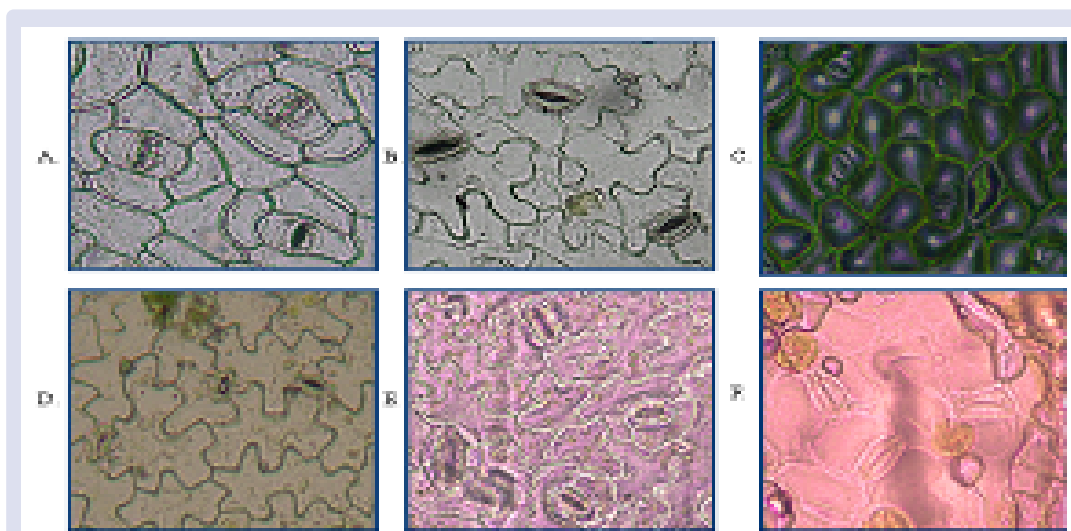

Figure 1: Lower epidermal cells of A. *Allocasia macrorrhiza* B. *Canavalia ensiformis* C. *Averrhoa carambola* D. *Amaranthus cruentus* E. *Capsicum Chinese* F. Bauhinia teniflora.
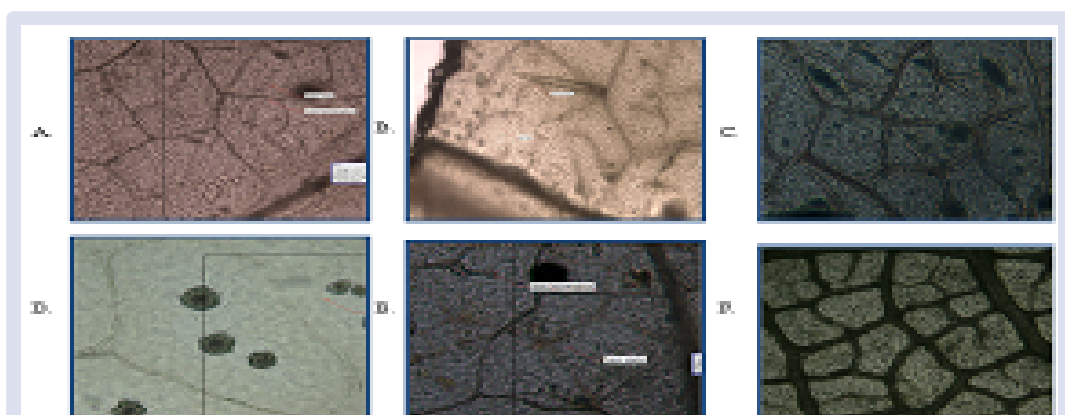
Figure 2: Vein islet and vein termination of A. *Allocasia macrorrhiza* B. *Canavalia ensiformis* C. *Averrhoa carambola* D. *Amaranthus cruentus* E. *Capsicum Chinese* F. *Bauhinia teniflora* (10X, 400µm sq.).
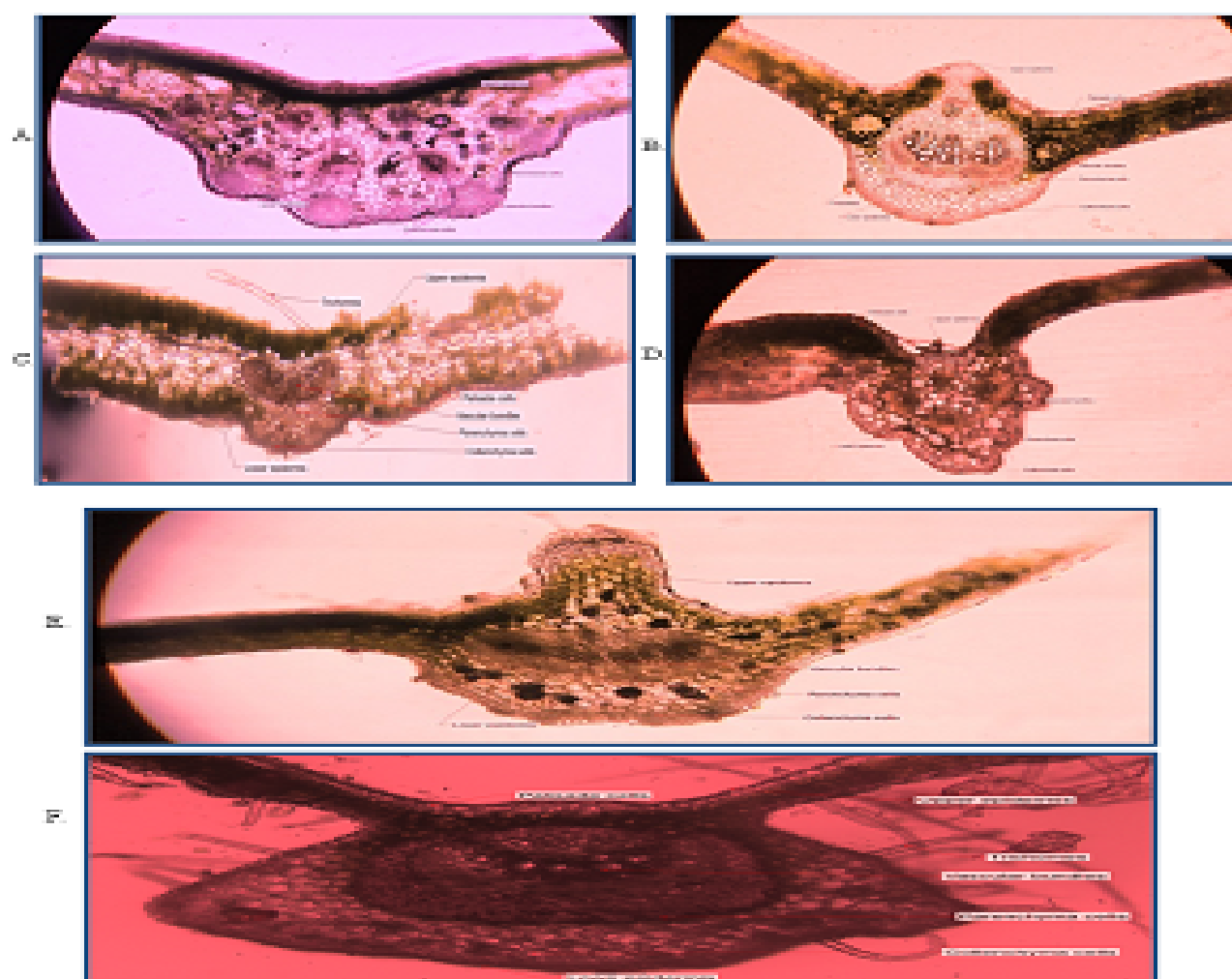
Figure 3: Transverse section of the leaves of A. *Allocasia macrorrhiza* B. *Canavalia ensiformis* C. *Averrhoa carambola* D. *Amaranthus cruentus* E. *Capsicum Chinese* F. *Bauhinia teniflora* (at 40X).
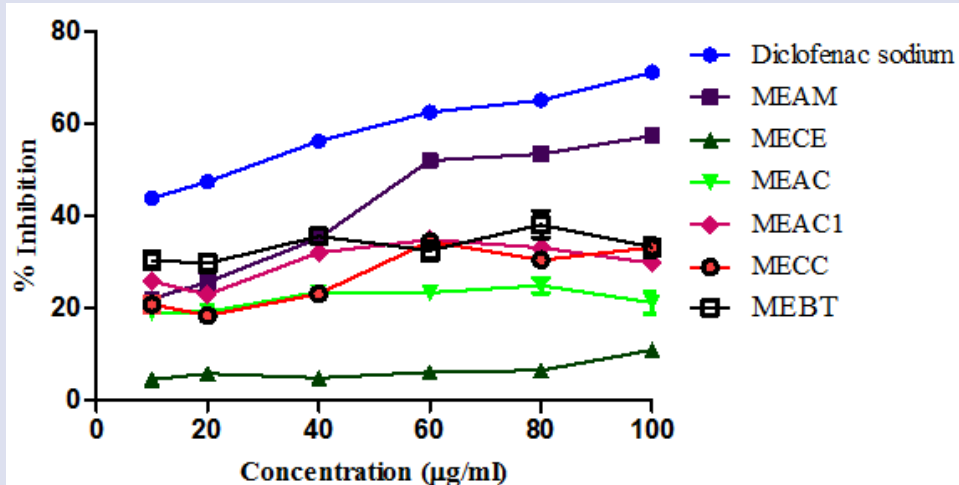
Figure 4: Comparison of concentration and % inhibition between methanolic extracts of *Allocasia macrorrhiza* (MEAM), *Canavalia ensiformis* (MECE), *Averrhoa carambola* (MEAC), *Amaranthus cruentus* (MEAC1), *Capsicum Chinese* (MECC), *Bauhinia teniflora* (MEBT) and standard drug (Diclofenac sodium).

subjected cold maceration using methanol as a solvent and screened for its anti-inflammatory responses. Inflammation is normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents.[31] From the synthetic medication Non steroidal anti-inflammatory drugs are commonly used for the management of inflammatory conditions, but has got many unwanted side effects such as gastric irritation, ulcer *etc.*[32] Medicinal plants used in traditional medicine to treat anti-inflammatory conditions seem a viable and logical alternative in search of safe and effective anti-inflammatory agents. The IC_{50} *value of MEAM, MECE, MEAC, MEAC1, MECC, MEBT against inhibition of protein denaturation was found to be 72.88, 841.78, 735.12, 303.75, 188, and 354.1855 μg/ml respectively. Out of six plants, methanolic extracts of Alocasia macrorrhizos showed potent anti-inflammatory activity while comparing with standard drug diclofenac sodium (IC_{50} -25.55μg/ml).* It is a well known fact that denaturation of tissue proteins lead to inflammatory and arthritic diseases.[33] Natural products that can prevent protein denaturation therefore, would be worthwhile for development of anti-inflammatory drug therapy. The pharmacognostical evaluation can thus give valuable information regarding its morphology, microscopic features and physicochemical characteristics which will help in the exact identification as well as assessment of purity of crude drugs.

RESULT

Collection and extraction of the plant material

The plants were collected from different parts of north east india, extraction was carried out by cold maceration, % yield of *Alocasia macrrorhizos*, *Canavalia ensiformis*, *Amaranthus cruentus*, *Averrhoa crambola*, *Bauhina tenifloara*, *Capsicum Chinese* was found to be 3.1, 3.9, 3.6, 4.5, 4.1 and 4.8 (%) respectively.

Pharmacognostic standardization assists in the correct identification, authentication and detection of adultery and routine quality control of crude drugs. Physicochemical numerical standards reported in this work will be useful for accurate identification, questionable model testing and compilation of a relevant monograph. Morphological examination examines the macroscopic features of crude drugs as depicted in table2.

Microscopic characteristics

Microscopy of leaves shows two subsidiary cells parallel to stoma. Hence it is paracytic type of stomata as seen in *Alocasia macrorrhizos*, *Averrohoea carambola*, *Capsicum chinese* while microscopy of the leaves shows the presence of anomocytic stomata as seen in *Canavalia ensiformis*, *Amaranthus cruentus*, *Bauhinia teniflora* as shown in Figure 1.
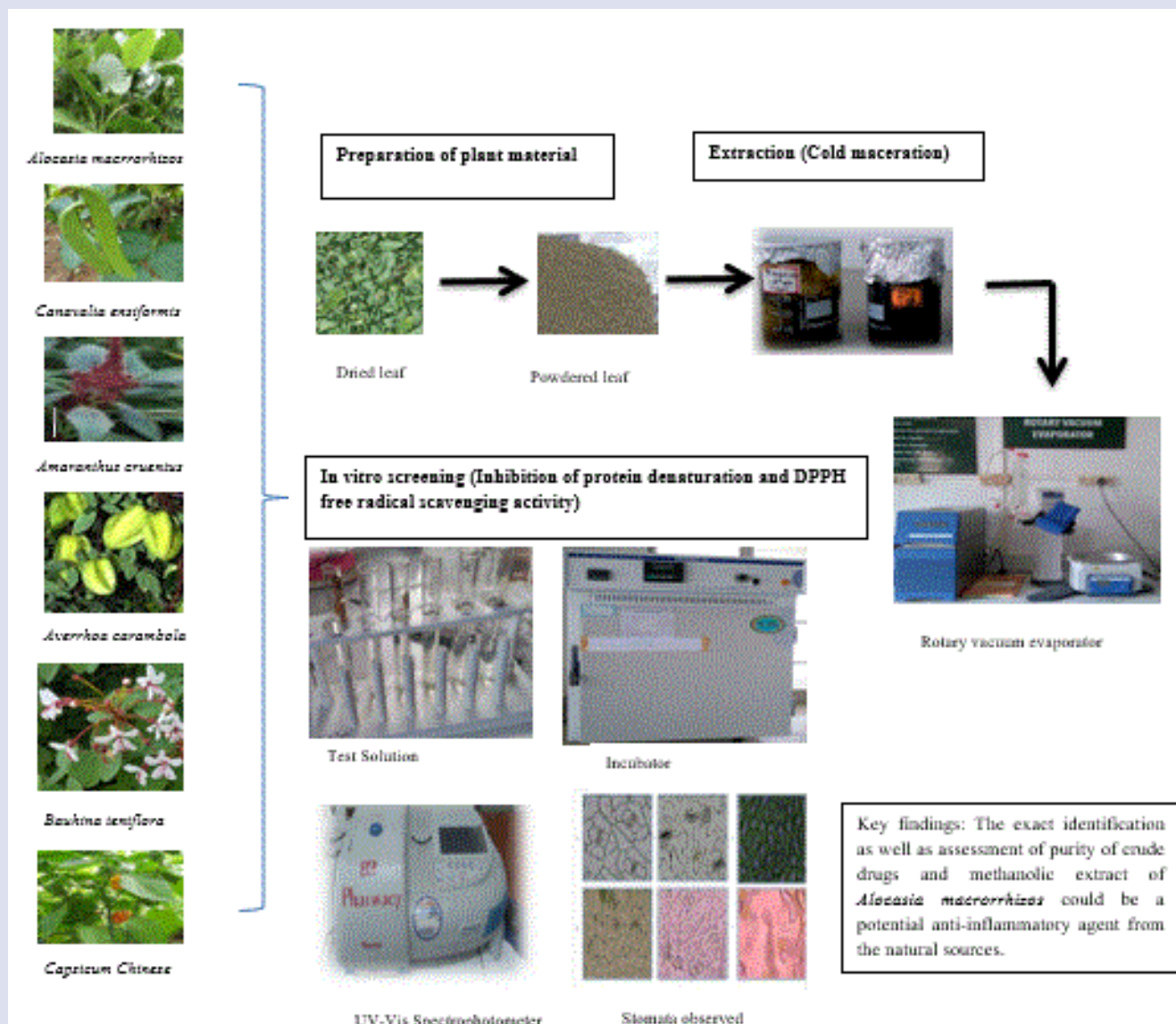
CONCLUSION

From our investigation, it can be concluded that majority of the information about the quality of crude drug can be obtained from the macroscopic analysis, microscopy, and physicochemical parameters. It could be useful tool for authentication of species and genus of plants and which enables us to ensure quality control of medicinal plants. *Alocasia macrorrhizos* could be a potent anti-inflammatory agent from a natural source.

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GRAPHICAL ABSTRACT

Alocasia macrorrhizos

Canavalia ensiformis

Amaranthus cruentus

Averrhoa carambola

Bauhinia teniflora

Capsicum Chinese

Preparation of plant material

Extraction (Cold maceration)

Dried leaf

Powdered leaf

In vitro screening (Inhibition of protein denaturation and DPPH free radical scavenging activity)

Test Solution

Incubator

Rotary vacuum evaporator

Key findings: The exact identification as well as assessment of purity of crude drugs and methanolic extract of *Alocasia macrorrhizos* could be a potential anti-inflammatory agent from the natural sources.

UV-Vis Spectrophotometer

Stomata observed

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