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FOURIER TRANSFORM-INFRARED (FT-IR) ANALYSIS AND PHYTOPHARMACOGNOSTIC SCREENING OF THE ARIEAL PARTS OF *CROTON BONPLANDIANUS*

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Abstract
Croton bonplandianus belongs to family Euphorbiaceae and it is an important traditional plants. Phytopharmacological analysis indicates that the leaves of *C. bonplandianus* significantly exhibited wound healing properties because of the presences of antioxidant enzymes and rutin etc. Present investigation comprises on organoleptic, microscopic, physicochemical and phytochemical analysis of aerial parts of this plant. Fourier Transform Infrared (FTIR) Spectrophotometer was also performed using leaves extract for the evaluation of peak value with various functional groups. Preliminary phytochemical screening was performed for the identification of medicinally valuable constituents. Fluorescence analysis was carried out to observe the behavior of powdered material with different reagents under UV light. All these parameters are used as tool for proper identification, authentication and standardization of plant material. Mid rib considerably curved upper epidermal and U-shaped lower epidermal cells while unicellular trichomes were short and branched at the base were exposed in histological assessment of the plant. Vascular bundle packed and conjoined. Wavy parenchymatous cells abundantly present with parasitic stomatas. The identical features of bark of *C. bonplandianus* were physically observed that exhibited wavy outline, barrel shaped, thick walled, compactly packed. Powdered leaves were revealed the presences of starch grain, stomata, parenchymatous cell and epidermal cells patches along with trichomes. Phytochemical studies indicated the presences of alkaloids, phenol, carbohydrates, steroids, saponins, proteins, and amino acids. FTIR analysis was conducted by spectrophotometric technique for the detection of functional groups and peaks values. FTIR spectrum profile of *C. bonplandianus* was generated for further estimation. This research would assist in future to reduces the chance of contamination, adulteration, improper &/or incorrect identification and purification, although supports the isolation of medicinally active constituents of *C. bonplandianus*.

Key words: *C. bonplandianus* bark and leaves, phytochemical, microscopic evaluation, physicochemical analysis, FTIR spectroscopy

INTRODUCTION

Medicinally valuable plants are universally utilized by human beings, due to the lesser side effects and the cost effectiveness comparative to marketed synthetic products. Drug obtained from the natural sources are deliberated to be harmless. Unfortunately due to the higher demand of herbal products and lack of data, it is difficult to identify the various medicinally valuable plants and their parts. Although the plants are safer, but owing to the contamination and adulteration as whole or in the powder materials may affect the response adversely. Pharmacognostic standardization and evaluation play significant role in the identification and purification of plant materials. Identification and standardization were elaborated in pharmacognostic analysis. Crude drugs exhibited a high safety profile with minimal side effects as demanded globally. According to the studies, WHO estimated that approximately 80% population have been utilizing plant materials for the prevention and treatment of several disorders of
lungs, heart, kidney, liver, skin, cancer, etc. [1 , 2]. Pharmacognostic evaluation deals with the identification, adulteration, morphology phytochemical and physicochemical investigation. These are significant components for standardization of a plant as whole or/and its parts for the eliminating the risk of contamination and adulteration [3 , 4 , 5].

Quality control measures support in minimizing the chances of error for the identification of crude drugs. Different parameters are utilized for the standardization of crude drugs to determine substitution or adulteration, foreign substances, microscopic and macroscopic evaluation, chromogenic testing, and ash value determination. WHO guidelines provided the scheme for the analysis of plant material through quality control measures [6 , 7 , 8]. Purity and the quality of herbal drug play a major role which may affect the potency and efficacy. Thus, not only the identification, quality and the purity of medicinally valuable plants are essential components of the formulation [9 , 10] . Bioactive constituents present in the plants were also be detected by several techniques such as FTIR spectroscopy, GC-MS spectroscopy, HPTLC etc.

FTIR is a powerful and reliable technique used for the identification of various functional groups present in plant extract. Specific absorption spectra displayed by different
chemical bonds are the fundamental feature [11 , 12 , 13].

ATERIALS AND METHODS

Collection and identification of plant C. bonplandianus

The leaves and barks of plant *C. bonplandianus* belong to the family Euphorbiaceae were collected from the University of Karachi and identified by Department of Botany (Taxonomy), University of Karachi, Pakistan in June 2018. A voucher specimen (no. 03) was issued and kept in the Department of Pharmacognosy, Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University (BMU), Karachi.

Chemical reagents and solvents

Glycerin (Merck, Germany), chloral hydrate (Merck, Germany), hydrochloric acid (Merck, Germany), absolute alcohol (Sigma Aldrich), clove oil, Canada balsam, iodine crystals, malachite green and safranin, □-naphthol solution, acetic acid, ammonium solution, benzene, chloroform, distilled water, ethanol, ether, hydrochloric acid, lead acetate, ninhydrin, picric acid, sulphuric acid, tannic acid and distilled water.

Chromogenic Reagents

Phytochemical evaluations were conducted using the coloring reagents such as Dragendorff's reagent and ferric chloride. Plants contain various chemical constituents such as alkaloids, flavonoids, terpenes,hydrocarbon, steroids, esters, etc., identified through different chromogenic reagents [14 , 15].

Apparatus and Instruments
Glass slides with cover slips, petri dish, needle, blades, brush, watch glasses, test tubes, Electronic Microscope B-350 (Optika microscope Italy), Grinder TSK-333 (WestPoint France), Gallen Kamp, hot oven Model: OVB 305 (UK).

Parameters for Standardization and Evaluation of Crude Drugs
Organoleptic evaluation Sensory organs such as sight, touch, smell and taste are involved in organoleptic evaluation that imitates the identity and purity thus assuring the quality of a particular plant material. Characteristic features of organoleptic analysis are based on shape, size, odor, color, taste, texture and fracture. Study of the leaves additionally based on leaf margin, apex, base, venation and inflorescences analysis [3 , 16].

Macroscopic and microscopic analysis
The study of morphology crude drug is labeled as macroscopic evaluation. This was usually done with naked eyes using magnifying glass [17 , 18]. Powder microscopy and histological examination of suitable section of plant material are basic elements of microscopic evaluation. Ethanol (different concentration), glycerin, iodine and chloral hydrate are different chemical reagents along with the malachite green and safranins stain dye materials. These assessments are used as major tools for the identification of crudedrug and for the analysis of purity with the help of distinguish structures [19 , 20].

P*hysicochemical *analysis Physicochemical analysis is comprise of loss on drying (LOD), moisturecontent (MC), total ash value, acid insoluble ash etc. [16 , 21].

Preliminary Phytochemical Analysis
Estimation of the quality and the purity of the crude drug is the foremost feature of pharmacognostic evaluation. The physicochemical and preliminary phytochemical evaluation was used for the quantitative studied as per standard protocols through various chromogenic reagents and also employed to evaluate the secondary metabolites too. Various chemical tests were carried out for the detection of different chemical constituents. Phytochemical analysis based on the identification of saponin, triterpenoids, alkaloids, carbohydrates glycosides, tannins etc. [22 , 23].

Histological evaluation
Histological studies were carried out to examine sequential organization of tissues and cells within the specimen. Bark and leaves of the plant *C. bonplandianus* were assessed for standardization of plant material [24].

Powder microscopy
Cleaned plant material were dried in hot air oven at 30-38ºC. Leaves were then ground through three blade grinder. Physical characters of powdered material were studied including as taste, odor, colour etc. Finally powdered material put on the slide and treated with the different reagents and coverslip was placed to observe under the microscope [25].

Fluorescence Analysis
Some of the powder material showed specific colour florescence that use in estimation of powder material and adulteration in the samples. Dry powder of plant material treated with the various chemicals reagents and placed in Ultraviolet (UV) cabinet to distinguish and identify the change in colour in ordinary and visible light at the short and long wave lengths [26].

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

For FTIR analysis dried powder of methanolic and aqueous extract of leaves and methanolic extract of bark of the plant *C. bonplandianus* were designated. Each powdered sample in each solvent were loaded in FTIR spectroscope in the range from 400-4000cm^{-1}[27].

ANTI-INFLAMMATORY ACTIVITY

MATERIALS

Acetic acid (0.2%)**,** diclofenac sodium, dimethyl sulfoxide (DMSO), distilled water, feeding tubes, Plethysmometer (Le 7500 Pan Lab Harvard Apparatus), syringes

METHOD

For the assessment of anti-inflammatory activity five animals per dose of each drug used against control group. 0.9% NaCl or DMSO (2%) and test samples were administered to the rats. MeOH extract of the bark of the plant *C. bonplandianus* (81, 54 and 27 mg/kg) were given to different groups of the rats. While positive control included diclofenac sodium (25mg/kg). For the evaluation of anti-inflammatory activity right hind paw were marked on ankle joint and paw edema was calculated three times using plethysmometer contemplate the interpretation as initial paw volume (Vo). 1% carrageenan (0.1 ml) was injected into paw of rat (right hind) at plantar region after 30 minutes of the administration of test samples and the standard drug (diclofenac sodium). The paw edema was noted at 1, 2, 3, 4, 5 and 6 hours demonstrating final paw volume (Vf). Percentage inhibition of the paw volume was calculated by Newbould method [25 , 28 , 29]. The results taken as the mean ± SEM and P-value were calculated to
estimate the statistical significant response using SPSS version 21 [30 , 31].

HISTOPATHOLOGICAL ANALYSIS

After performing the anti-inflammatory activity animals were sacrifice using standard protocol. Right hind paws of each animal were removed and fixed in buffered formalin for histopathological analysis [32 , 33]. A standard method were adopted followed by proper cleaning and fixation, slides were stained with hematoxylin and eosin (H & E stained) of all samples. Then labeled and studied for various morphological parameters to observed morphological and pathological changes [34 , 35].

RESULTS

The results of pharmacognostic assessment based on organoleptic evaluation and powder microscopy of the leaves of *C. bonplandianus* are mentioned below.

Characteristic Features of Leaves C. bonplandianus

Distinctive features of the leaves of *C. bonplandianus* are presented in (Table 1). Results of histological examination of fine section of leaves and bark under microscope were demonstrated in figure 1a&b respectively.

Table 1.Distinguish Characteristics of Leaves of *C. bonplandianus*

	Characteristic features of leaves
Texture	Uneven
External Marking	simple, alternate, petiolate, cladodromous Venation, lamina obtuse lance-ovate, base
Margin	
Internal Marking	not prominent
Fracture	Soft and fibrous
Shape	Lanceolate
Size	2.6-4.5 X 1.5-2 cm
Odour	Not characteristic
Colour	Green
Taste	Slightly bitter

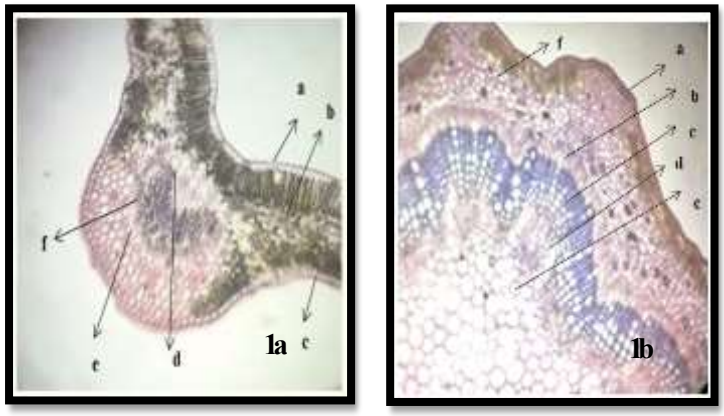


Fig. 1a. Transverse section of leaves of *C. bonplandianus* (a) Upper epidermis; (b) stomata with cuticle; (c) Lower epidermis; (d) Phloem vessel; (e) Xylem vessel; (f) Pericycle fibre; Fig1b. Transverse section of bark of C. bonplandianus (a) epidermis; (b) cambium; (c) Phloem; (d)) Xylem vessel; (e) pith; (f) cork

Powder Microscopy of Plant C. bonplandianus Leaves

Powder microscopy of the leaves of *C. bonplandianus* was carried out using the different reagents including iodine solution (5%), aqueous glycerin solution (50%) and chloral hydrate (10%). Predominant cellular features are observed under microscope described in (Table 2 & Fig.2&3)

Table 2. Powder microscopy and Characteristic Features of powdered leaves of C. bonplandianus

No.	Characteristic features of powdered drug		Observation in different reagents		
			10% chloral hydrate	5% iodine solution	50% aqueous glycerin solution
1.	Parenchyma	Wavy parenchymatous cells abundant present with parasitic stomatas	+++	+++	++
2.	Starch Grain	Spherical compound and simple starch grains	–	+	++
3.	Stomata	Paracytic, surrounded by 2 subsidary cell. Stomatal pore elliptical in outline	+++	+	++
4.	Cork Tissues/ cells	Rectangular in shape, thick and lignified	+	++	+++
5.	Ca-Oxalate crystals	Multisided prismatic crystals of Ca-Oxalate abundantly founded	+++	+++	+
6.	Vascular bundle	Single, conjoint, collateral, endarch and closed	+++	++	+
7.	Group of Fibre with sheet of cork-tissues	Present	++	+++	+++
8.	Oil cells	Scattered large thin-walled oil cells	++	+	+++
9.	Trichomes	Stalked, glandular, unicellular, short, branched at base and arises from lower epidermis	+++	+++	++

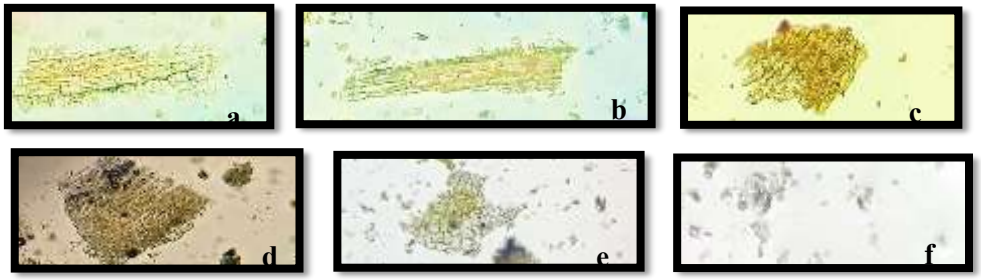


Fig. 2 a& b. Group of Fibres with sheet of cork-tissues and parenchymatous cells; c&d. Parenchymatous cells with epidermis; e & f. Stomata showed as Paracytic, with starch grain

Fig. 3 a,b &c. Stalked, glandular, unicellular, short, branched at base and arises from lower epidermis

Phytochemical Screening for Quality Control analysis: The phytochemical investigation was showed that presences of different chemical constituents in methanolic bark and leaves extract of the plant C.*bonplandianus* (Table 3).

Table 3. Phytochemical screening of methanolic extracts of C. *bonplandianus* Physicochemical Analysis

No.	Test	Leaves extract	Bark extract
1.	**Tannin**		
a.	**Ferric chloride test**	Olive green color	Olive green color
b.	**Lead acetate test**	Moderate precipitation	Moderate precipitation
2.	**Anthraquinones**	No pink color	No pink color
3.	**Saponins**	Frothing not persistence	Frothing not persistence
4.	**Flavonoids**	Pink tomato red color	Pink tomato red color
5.	**Terpenoids / Steroids**	Light grey color	Grayish green color
6.	**Alkaloids**	orange precipitation	yellowish orange precipitation
7.	**Carbohydrates**	Purple color	Purple color
8.	**Ketones**	Green color	Olive-green to brown color
9.	**Glycosides**		
a.	**Salkowski's test**	Brown color	Reddish-brown colour
b.	**Liebermann's test**	Dark green colour	Violet colour

Powder behavior of leaves *C. bonplandianus*: Powdered leaves of the *C. bonplandianus* are displayed different colors when treated with various chemical reagents (Table 4).

Table 4. The behavior of powdered leaves of *C. bonplandianus* with different chemicals

No.	Treatment with chemicals	Ordinary light
1.	Powder triturate with water	Emulsion not formed
2.	Powder shake with water	Absence of froth
3.	Powder treated with 5 % NaOH	Yellowish green color
4.	Powder treated with 5% FeCl3	Green color
5.	Powder treated with 66 % H2SO4	No effervescent appear
6.	Powder press between two filter paper (24 hours)	No changed or stain

Fluorescence analysis: Fluorescence analysis was conducted for the powdered material of the leaves of *C. bonplandianus* and outcomes are demonstrated in Table 5.

Table 5. Fluorescence Analysis of powdered leaves of *C. bonplandianus*

No.	Treatment with Solvent	Ordinary light	Short light (254 nm)	Long light (366 nm)
1.	Powder as such	Yellowish green	Green	Dark green
2.	Powder with 1N NaOH	Green	Green	Dark green
3.	Powder with 50% H2SO4	Green	Dark green	Dark green
4.	Powder with 50% HCl	Yellowish green	Green	Dark green
5.	Powder with CH3COOH	Yellowish green	Green	Dark green
6.	Powder with HNO3	Yellowish-brown	Yellowish green	Brown

Physiochemical screening (Ash content): Physicochemical analysis was carried out using different parameters including total ash, water-insoluble ash, suphlated ash, acid-insoluble ash, LOD, dry matter weight. The result of physiochemical screening is elaborated in Table 6.

Table 6. Physiochemical screening of powdered leaves of *C. bonplandianus*

No.	Parameters	(% w/w)
1.	**MC**	4.8%
2.	**LOD**	5.17%
3.	**DM**	94.2%
Ash values		
4.	**Total ash**	17.5%
5.	**Acid insoluble ash**	1.2%
6.	**Water insoluble ash**	1.45%
7.	**Sulphated ash**	11.8%

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: The data on the peak values and the possible functional groups obtained by FTIR analysis present in the aerial part of *C. bonplandianus* were presented (Table 7-9 and Fig. 4-6) below.

Table 7. FTIR analysis of powdered methanolic leaves extract of C. bonplandianus

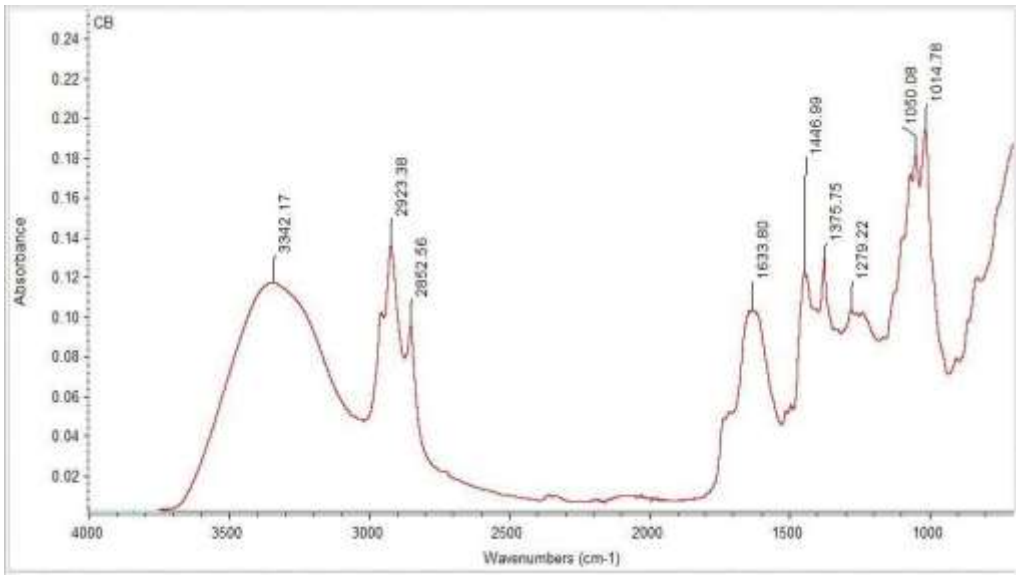
Peak values	Functional group
1014.78	Aliphatic amines
1050.08	C-O group /secondary Alcohol
1279.22	C-N band/amide
1375.75	C-H bending/aliphatic group
1446.99	C=O streching/carboxylic acid/ aromatic
1638.80	C=C group / N-H/ primary amines
2852.56	C-H group / Alkanes / CH and CH2 stretching/aliphatic group
2923.38	C-H stretching/ CH and CH2 stretching/aliphatic group
3342.17	O-H stretching/ Amines/ alcohol

Table 8. FTIR analysis of powdered aqueous leaves extract of *C. bonplandianus*

Peak Value	Functional group
1048.66	C-N stretching/ Aliphatic amines
1382.50	C-H bending/ Aldehyde
1617.26	C = C steching/ alkene
3319.33	O-H stretching/ Alcohol

Table 9. FTIR analysis of powdered methanolic bark extract of *C. bonplandianus*

Peak values	Functional group
863.67	C=O bending/ inorganic carbonate/Primary, secondary amines
905.42	Aliphatic amines
1047.66	C-O bonding/ether/Aliphatic amines
1377.43	C-H bending/aliphatic group
1616.94	C = C group/ primary amines/unsaturated compounds
2924.86	CH and CH2 stretching/aliphatic group
3295.27	C-H stretching



Fi.g 4. FTIR analysis of powdered methanolic leaves extract of *C. bonplandianus*

Fig. 5. FTIR analysis of powdered aqueous leaves extract of *C. bonplandianus*

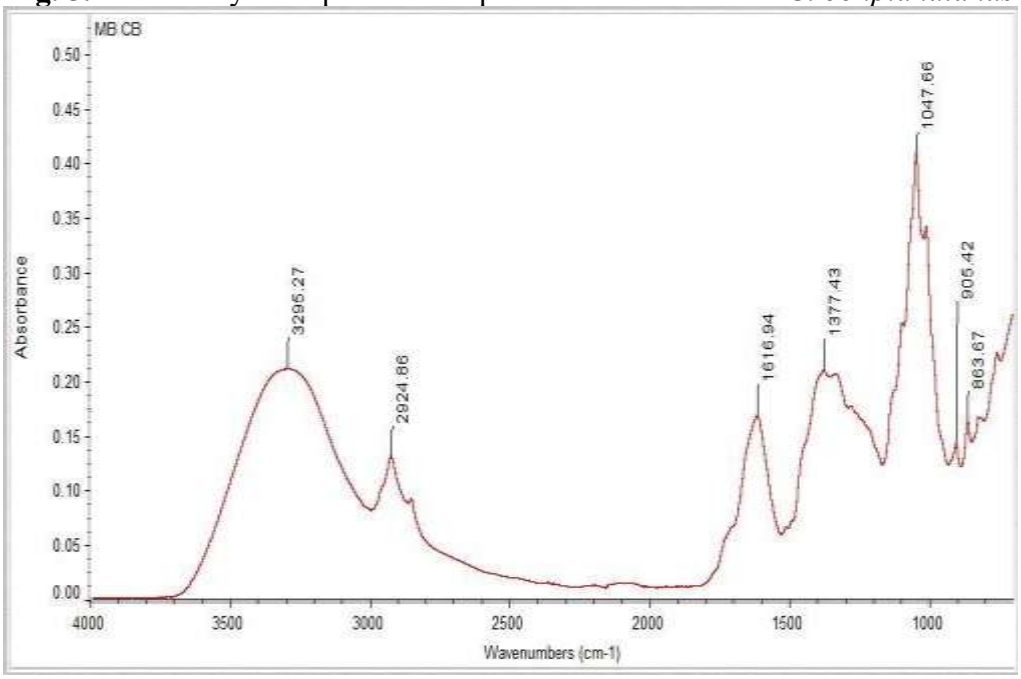


Fig. 6. FTIR analysis of powdered methanolic bark extract of *C. bonplandianus*

Table 2. Anti-inflammatory Activity of the Bark Extracts of *C. bonplandianus*

Groups	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
C	2.14±0.13	3.28±0.10	3.48±0.06	3.65±0.04	4.12±0.16	4.27±0.02	4.61±0.13
DS	2.78±0.12	3.27±0.15	3.93±0.17	3.80±0.21*	3.65±0.10**	3.57±0.16***	3. 48±0.13***
MB-81	3.39±0.04	3.89±0.25	4.20±0.07	4.18±0.02	3.93±0.13*	3.79±0.03*	3.41±0.10*
MB-54	3.16±0.05	3.73±0.04	3.97±0.60	3.81±0.02*	3.71±0.02*	3.64±0.04**	3.44±0.01**
MB-27	3.81±0.05	4.35±0.04	4.51±0.04	4.43±0.03*	4.30±0.05*	4.10±0.01***	4.02±0.08***

Where: C= positive control, DS= Diclofenac sodium, MB = methanolic bark extract of *C. bonplandianus* (81, 54, 27 mg/kg).The data were subjected to statistical analysis using one-way analysis of variance (ANOVA). P values < 0.05 were considered significant.Significant at $^{*}P < 0.05$, $^{***}P < 0.01$, $^{***}P < 0.001$ when compared to control (n = 3).

Table 3. Percentage Inhibition of Paw Volume

Groups	% Inhibition of paw volume at different time intervals (hours)					
	1	**2**	**3**	**4**	**5**	**6**
DS	14.89	22.41	58.33	82.60	90.28	92.82
MB 81	8.5106	48.276	16.6667	50.7246	76.5714	95.2153
MB 54	19.149	36.207	32.2917	60.1449	73.1429	91.866
MB 27	17.021	22.414	34.375	70.2899	79.4286	84.689

Where; DS= Diclofenac sodium, MB=methanolic bark extract of *C. bonplandianus* (81, 54 and 27 mg/kg)

Histopathological studies of rats paw

Figure 1

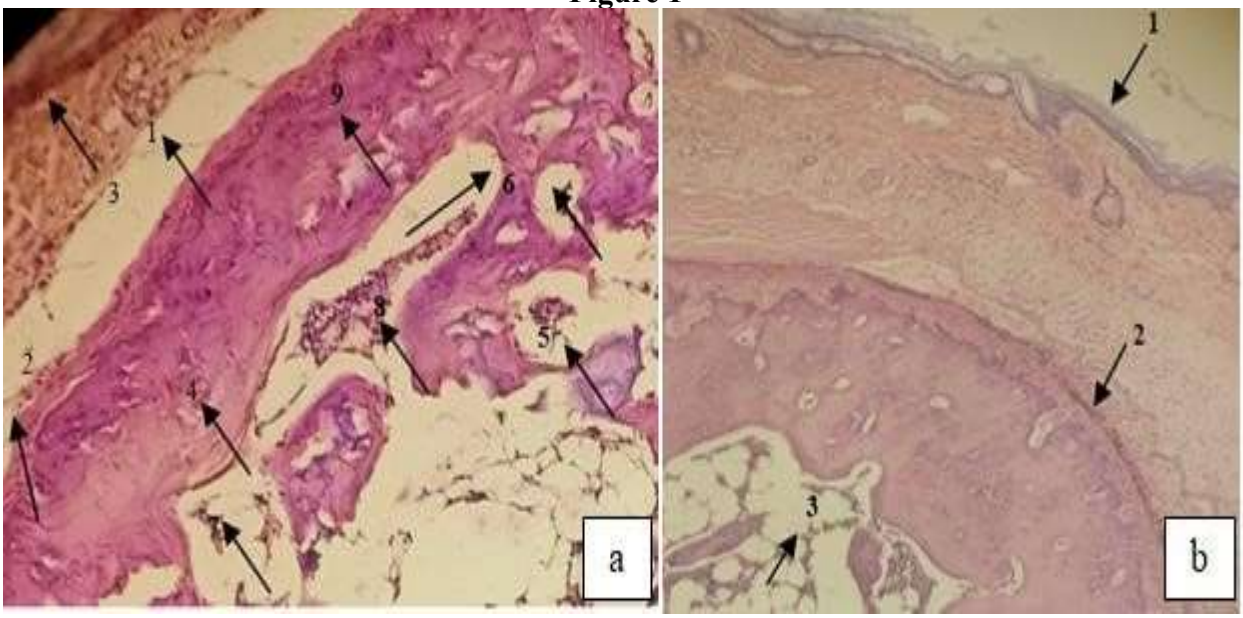


Fig.6.4: Photomicrograph of 5 micron thick H & E stained paraffin section from right hind paw of rats of **a :control group** showing; 1= epidermis uplift, 2=Bolus,3=dermis, 4=vasodilation, 5= Hemorrhages, 6=edema, 7= Collagen fiber, 8=Plasma cells, 9=Mucoidal swelling. **b: DS (standard drug)** 1= Dermis, 2= Vasodilatation, 3= Edema

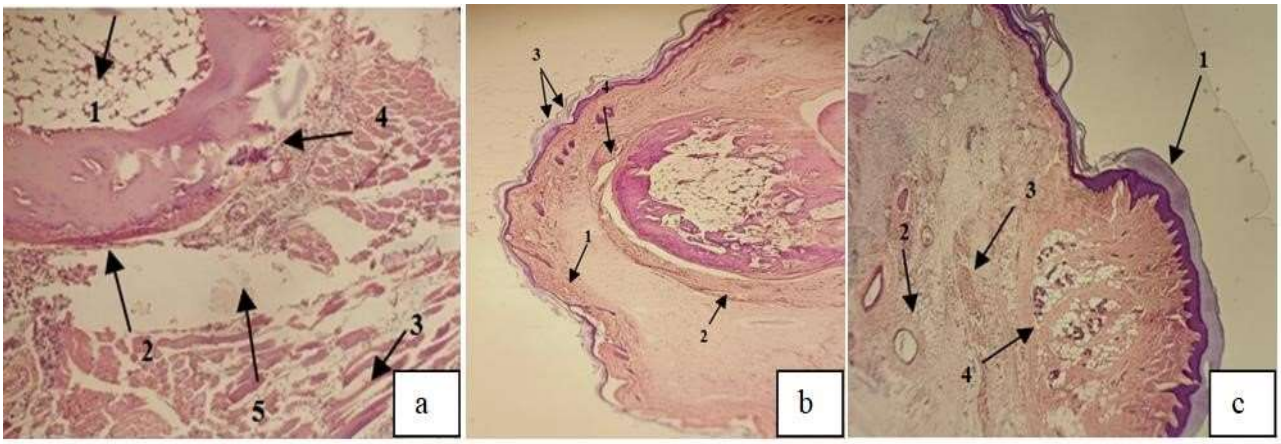


Fig .6.7: Photomicrograph of 5 micron thick H & E stained paraffin section from right hind paw of rats showing **a: MB-81**: 1=Collagen fiber, 2= Vasodilation, 3= Hemorrhages, 4= Plasma cell, 5= Edema. **b: MB-54**: 1= Dermis, 2= Vasodilation, 3=Keratin, 4= Inflammation. **c: MB-27:**1= Epidermis, 2= Collagen Fiber, 3= Mild vasodilation, 4= Inflammation

Discussion

Herbal plants indeed played a vital role in the healthcare system for centuries. Medicinally valuable plants contribute a fundamental part to the development of novel compounds that are effectively used
therapeutically to treat various ailments [36 , 37]. World Health Organization (WHO) studied that approximately 75-85% population of the world in developing countries used herbal plants as medicine rather than modern medicines to due lack of accessibility and side effects. According to research 2530% of medicine is derived from plant materials either by direct or indirect means. Compared with modern drugs, medication originated from the plants material attributed to their safety profile owing to lesser adverse effects and comparatively marked therapeutic activity at the lowest cost [38].

Based on successive investigations, medicinal plants are flourishing with demand internationally. However the chance of the substitution and the adulteration with the standard plant
material might be originated [39 , 40]. Besides, the adulteration and contamination with toxic or harmful matters like heavy metals, pesticides, inorganic salts, and microbes in crude drugs as a part or whole are not appropriate to be used safely. So, the identification and the standardization of therapeutically
valuable plants are desirable for the maintenance of quality therapeutic efficiency [41 , 42]. Identification and standardization of crude drug are the prime tools for investigation through pharmacognostic evaluation. This assessment is comprised on morphological, organoleptic evaluation and microscopical examination of plant materials. Official pharmacopoeias have elaborated the diagnostic
pharmacognostic features of various valuable plant materials for their proper identification [43 , 44 , 45].

Current investigation was performed on dried leaves and bark of the plant *C. bonplandianus* (Euphorbiaceae) synonym is *Croton sparsiflorus* locally known as “ban tulsi”. *C. bonplandianus* is a perennial herb indigenous to southern Bolivia, Paraguary, northern America and south western Brazil
however also cultivated in India, West Bengal and Pakistan [46 , 47 , 48].

Organoleptic and microscopic examination was implemented for preliminary evaluation. The leaves of the plant *C. bonplandianus* are green with lance-ovate lamina, simple, alternate, petiolate, cladodromous venation with base obtuse, abaxial side light green and pubescent while dark green and glabrous from adaxial side. Trichomes are unicellular and short, undistinguishable cortex and parenchymatous cells, oil cells and vascular bundles are present (Table 1). Histological examination of leaves of *C. bonplandianus*, mid rib showed considerably curved upper epidermal and U-shaped lower epidermis cells. Trichomes appeared as unicellular, short and branched at the base. Vascular bundle was seemed closely packed and conjoined. Wavy parenchymatous cells abundantly present with parasitic stomatas (Fig. 1a). Transverse section of bark displayed wavy margin having short trichomes. Chlorenchyma and parenchyma cells present alternatively while middle cortex showed the presences of sclerenchymatous patches. The identical features of bark of *C. bonplandianus* were physically observed that showed wavy outline, barrel shaped, thick walled, compactly packed (Fig. 1b).

Assessment of the powder characteristic of the leaves of *C. bonplandianus* were carried out by powder microscopy with chloral hydrate (10%), aqueous glycerin (50%) and iodine (5%) and (Fig. 2&3). Powdered leaves were shown stomata, parenchymatous cell and epidermal cells patches along with trichomes. Prism like calcium oxalate crystal, stone cell and starch granules were also founded. Starch grain clearly observed in iodine solution (5%) while stomata, fibers, cork cell and trichomes were visible in 10% Chloral hydrate solution and aqueous glycerin 50% (Table2). For the identification and standardization of crude drug above mentioned observation provided an authentic results for assessing
the quality and purity content for contamination, substitution and adulteration etc. [28 , 49].

The preliminary phytochemical and physiochemical evaluations were conducted for the leaves and bark of the plant of *C. bonplandianus*. Physiochemical analysis based on powdered behavior with the various chemicals (Table 4), and fluorescence analysis of powdered leaves (Table 5). Physiochemical parameters included LOD, MC, DM, and determination of ash value including total ash value, acid insoluble ash, water-insoluble ash, and sulfated ash (Table 6) [50] .

The leaves of the plant of the *C. bonplandianus* contained MC 4.8%, DM 94.2%, and exhibited 5.17% loss on drying. Research studies revealed that the presence of moisture in powder susceptible to the fungal attack and may lead to the deterioration of the various secondary metabolites and the loss of the therapeutic activity because of the chemical alterations. Presence of the moisture may cause the activation of enzymes and microorganism growth. However in this plant moisture content (MC) 4.8% was founded to acceptable limits according to WHO guild lines and other the official pharmacopeia that
assure the plant is safely used as medicine in the future [22 , 51 , 52]. Ash value is one of the foremost parameters to evaluate the adulteration and contamination in powdered material [53]. Ash value based on the total ash value, acid insoluble ash, water-insoluble ash, and sulphated ash were demonstrated in Table 6.

Fluorescence behavior of the powder material was detected when treated with the different chemical reagents under the ordinary light, short (254 nm), and long (366 nm) UV light that presented several colors (Table 5). For the quality analysis of the powder material, fluorescence behavior showed a significant part in the evaluation of the quality and the purity of various plant materials as they behave differently. The presence of chromophoric components in the several compounds showed fluorescence only in UV light when treated with different chemical reagents these components may be deteriorating
and/or formed fluorescent derivatives [3 , 54].

Phytochemical studied of the methanolic extracts leaves and bark of *C. bonplandianus* were accomplished for the evaluation of different chemical constituents like alkaloids, phenol, carbohydrates, steroids, saponins proteins, and amino acids (Table 3). Saponins, amino acids, and anthraquinones have not founded in both bark and leaves. Flavonoids were present in both methanolic extracts which indicates that the plants may possess immunomodulatory and antioxidant properties which may be used
to treat cancer, inflammation, allergy, microbial infections, and other disorder [55]. Ketones and terpenoids were detected in both extracts while glycosides were found only in bark abundantly. Alkaloids and tannins were specified in methanolic bark and leaf extracts of *C. bonplandianus*. Hence,
the plant was used for antibacterial, antifungal, anthelmintic, and wound healing activities [56 , 57]. Alkaloids are the major class of secondary metabolites that have various pharmacological activities including antioxidant, muscle relaxant, and analgesic properties. Alkaloids are indicated to treat various life-threatening diseases in human beings [58 , 59].

Recorded FTIR spectra of methanolic and aqueous extracts of *C. bonplandianus* were indicated characteristic absorption band that were displayed in Table 7-8, figure 4&5. Methanolic extract of bark of *C. bonplandianus* exhibited characteristic band expressed in table 9, figure 6. The FTIR spectral analysis of plant *C. bonplandianus* of the leaves and bark reported the presence of characteristic functional groups aliphatic amine, primary, secondary amines, carbonyl group, alcohol derivative, carbonyl group, aromatic compounds that will be responsible for several therapeutic activities [60] .

Anti-inflammatory activity

The historical consequence of herbs in traditional medicine effectively employed for the treatment of several ailments. One of the major causes of various diseases and its associated symptoms are inflammation. Most of the plants as whole and/or a parts were utilized as anti-inflammatory medicine as

producing more responses even than standard anti-inflammatory drugs [61 , 62]. Methanolic and aqueous extracts of the leaves and methanolic extract of the bark of the *C. bonplandianus* were selected for the assessment of the anti-inflammatory activity by carrageenan induced paw edema against standard antiinflammatory drug diclofenac sodium. Diclofenac sodium is classify as effective non-steroidal antiinflammatory drug (NSAID) that inhibit the leukocyte and cyclooxygenase enzyme system (Cox-1 and Cox-2), thus causes peripheral inhibition of prostaglandin [63]. For the evaluation of anti-inflammatory effects of natural product carrageenan-induced paw edema is an absolute model. Anti-inflammatory activity was conducted in different groups of adult albino rats wistar strains (200±10 g) with methanolic extract of bark of *C. bonplandianus* and results were given in table II and III. Results revealed the dose dependent anti-inflammatory response to carrageenan induced paw edema (Fig. 2 and 3) [64].
Anti-inflammatory activity along with histopathological studies was conducted for the methanolic extract of bark of the *C. bonplandianus*. All treated groups possessed significant dose dependent anti-inflammatory response with marked reduction in inflammatory mediator with rat's paw (Fig. 4-7).
It has proven by previous researches that the plants extracts and/or isolated compounds play significant role in the treatment of various disorders associated with inflammation [65]. Thus the plants play significant role in inflammatory problem, probably due to the presence of phenolic compounds. Polyphenolic compound in the plant responsible to provide nutrition in the human beings and it is well documented that the dietary phenolic compounds exhibited anti-inflammatory, antioxidant, anticancer properties [66]. Naturally isolated flavonoids and terpenoids also possess antioxidant, anticancer and antiinflammatory effects [67]. It is proposed that the *C. bonplandianus* might contain high amount of ploy phenolic compounds, flavonoids and terpenoids that would further confirmed by phytochemical analysis.

Histopathological analysis is sophisticated technique used to estimate the change in overall architecture of the cellular organelles. Histopathological evaluation also ensure the safety of drugs employed for the treatment of many diseases [64]. Right hind Paw of all treated rats were exposed to histopathological analysis and results are illustrated in photomicrographs (Fig. 4-7). Neutrophils infiltration within the cells is the characteristic feature of carrageenan induced inflammation[68] . On the behalf of histological finding, it has proven that in comparison to all groups, the maximum doses of methanolic extract of the bark of the *C. bonplandianus* elicited marked reduction in inflammation and neutrophils infiltration in rats paw ((Fig. 4-7).

Bark of *C. bonplandianus* in higher dose could be safely and effectively used as anti-inflammatory agent as proved through histopathological screening.
This research study provides a tool for investigation regarding the standardization of the different extracts and identification of the plant material. *In-vitro* and *in-vivo* studies of the plants and isolated constituents will also perform in future to improve outcomes. Pharmacological screening of the naturally isolated pure compounds might be used for the development of new, more effective, potent and safer medication.

Conclusion

Inclusive physiochemical and phytochemical analysis would aid in the proper identification and preventing the chance of contamination, adulteration, and substitution in various steps of isolation and separation. Moreover, the present study also helps for the detection of medicinally valuable secondary metabolites from the leaves and bark of the plant of *C. bonplandianus.*

Conflict of interest

There is no conflict of interest.

REFERENCES

1. Jamshidi-Kia F., Lorigooini Z., Amini-Khoei H. Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology.**7**(1). 2018
2. Ylla G., Liu T., Conesa A. MirCure: a tool for quality control, filter and curation of microRNAs of animals and plants. Bioinformatics.**36**(Supplement_2):i618-i24. 2020
3. Rashid S., Zafar M., Ahmad M., Lone F.A., Shaheen S., Sultana S., et al. Microscopic investigations and pharmacognostic techniques used for the standardization of herbal drug Nigella sativa L. Microsc Res Tech.**81**(12):1443-50. 2018
4. Fernandes F.H.A., Boylan F., Salgado H.R.N. Quality standardization of herbal medicines of Spondias dulcis Parkinson using analytical and microbiological analysis. Journal of Thermal Analysis and Calorimetry.**134**(3):1923-8. 2018
5. Rausch H., Schröder S., Friedemann T., Cameron S., Kuchta K., Konrad M. History and Present of European Traditional Herbal Medicine (Phytotherapy). History, Present and Prospect of World Traditional Medicine: World Scientific; 2024. p. 131-234.
6. Prajapati N.D. Handbook of medicinal plants: Agrobios; 2003.
7. Prajapati N.D., Purohit S., Sharma A., Kumar T. Medicinal plants. Agrobios published company, 3rd edition, India.**353**. 2003
8. Organization W.H. Quality assurance of pharmaceuticals: a compendium of guidelines and related materials. Volume 2. Good manufacturing practices and inspection: World Health Organization; 2024.
9. Dharmadasa R., Samarasinghe K., Adhihetty P., Hettiarachchi P. Comparative Pharmacognostic Evaluation of Munronia Pinnata (Wall.) Theob.(Meliaceae) and Its Substitute Andrographis paniculata (Burm. f.) Wall. Ex Nees (Acanthaceae). 2020
10. Jugran A.K., Rawat S., Bhatt I.D., Rawal R.S. Valeriana jatamansi: An herbaceous plant with multiple medicinal uses. Phytother Res.**33**(3):482-503. 2019
11. Khan S., Shinwari M.I., Haq A., Ali K.W., Rana T., Badshah M., et al. Fourier-transform infrared spectroscopy analysis and antifungal activity of methanolic extracts of Medicago parviflora, Solanum Nigrum, Melilotus alba and Melilotus indicus on soil-borne phytopathogenic fungi. Pakistan Journal of Botany.**50**(4):1591-8. 2018
12. Nawaz F., Ahmad S., Naheed S., Ahmad Z., Mehmmod T. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of Monotheca Buxifolia. University of Wah Journal of Science and Technology (UWJST).**3**:15-22. 2019
13. Baishya T., Das P., Ashraf G.J., Dua T.K., Paul P., Nandi G., et al. Antioxidant activity, cytotoxicity assay, and evaluation of bioactive compounds using GC-MS and HPTLC-based bioassay in the extracts of Osbeckia stellata var. crinita (Benth. ex Naudin) grown in Manipur, India. Kuwait Journal of Science.**51**(3):100229. 2024
14. Guo Y., Han Z., Zhang J., Lu Y., Li C., Liu G. Development of a high-speed and ultrasensitive UV/Vis-CM for detecting total triterpenes in Traditional Chinese Medicine and its application. Heliyon. 2024
15. Madhusudhan M., Sushma T., Gulnaz A., Gowda A.C., Roopa G., Pushpalatha H., et al. Evaluation of phytochemical composition and in-vitro assessment of antioxidant and antimicrobial activities of various medicinal plant extracts. Journal of Pharmacognosy and Phytochemistry.**13**(3):37-45. 2024
16. Fatima K., Mahmud S., Yasin H., Asif R., Qadeer K., Ahmad I. Authentication of various commercially available crude drugs using different quality control testing parameters. Pak J Pharm Sci.**33**(4). 2020
17. Khadhraoui B., Turk M., Fabiano-Tixier A., Petitcolas E., Robinet P., Imbert R., et al. Histo-cytochemistry and scanning electron microscopy for studying spatial and temporal extraction of metabolites induced by ultrasound. Towards chain detexturation mechanism. Ultrason Sonochem.**42**:482-92. 2018

18. Sarfraz M., Alam F., Din K.M., Malik S., Quddoos A. Pharmacognostic characterization of Dicleptera chinensis by scanning electron microscopy, light microscopy, and analytical techniques. Microsc Res Tech.**87**(2):279-90. 2024
19. Singh D., Aeri V., Ananthanarayana D. Development of internet technology TIPHAM (Tool for identity of Powdered Herbals through Analytical Microscopy) for microscopic identification of crude herbal drugs. Pharmacogn Mag.**14**(55):213. 2018
20. Joseph T., Simon T.K. ORGANOLEPTIC AND POWDER CHARACTERISTICS AS TOOL FOR ANDROGRAPHIS SPECIES IDENTIFICATION. 2019
21. Sharma P.K., Mansoori S. Quantitative and Qualitative Analysis of Zingiber officinale as A Crude Drug. Research Journal of Pharmacy and Technology.**12**(5):2157-9. 2019
22. Kokate C. Preliminary phytochemical screening. Practical pharmacognosy.106-11. 2000
23. Devi M.R., Krishnakumari S. Quantitative estimation of primary and secondary metabolites in hot aqueous extract of Pleurotus sajor caju. Journal of Pharmacognosy and Phytochemistry.**4**(3):198. 2015
24. Kaur G., Rubal K., Srivastava D.K., Singh P.K., Ahluwalia A.S. Morpho-histological study in the gynodioecious Salvia strobilifera (Lamiaceae). Vegetos.1-12. 2024
25. Khan A., Ahmad M., Sultan A., Khan R., Raza J., Ul Abidin S.Z., et al. Herbal Spices as Food and Medicine: Microscopic Authentication of Commercial Herbal Spices. Plants.**13**(8):1067. 2024
26. Ranjith D. Fluorescence analysis and extractive values of herbal formulations used for wound healing activity in animals. J Med Plant Studies.**6**(2):189-92. 2018
27. Ramya S., Loganathan T., Chandran M., Priyanka R., Kavipriya K., Pushpalatha G.G.L., et al. Phytochemical Screening, GCMS, FTIR profile of Bioactive Natural Products in the methanolic extracts of Cuminum cyminum seeds and oil. Journal of Drug Delivery and Therapeutics.**12**(2-S):110-8. 2022
28. Yasin H., Khalid S., Abrar H., Rizwani G.H., Perveen R., Fatima K. Comperative phyto toxicological and anti inflammatory effects of leaves extracts of holoptelea integrifolia. Bangladesh Journal of Medical Science.**17**(2):212-7. 2018
29. Newbould B. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. British journal of pharmacology and chemotherapy.**21**(1):127-36. 1963
30. Mansouri M.T., Hemmati A.A., Naghizadeh B., Mard S.A., Rezaie A., Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian Journal of Pharmacology.**47**(3):292. 2015
31. Noorian P. Evaluation of Gallic Acid anti-inflammatory effect on PGE2 and COX-2 in carrageenan induced rat paw edema: Mazandaran University of Medical Sciences; 2020.
32. Yasin H., Mahmud S., Rizwani G.H., Perveen R., Abrar H., Fatima K. Effects of aqueous leaves extract of Holoptelea integrifolia (Roxb) Planch on liver and kidney histopathology of albino rats. Pak J Pharm Sci.**32**(2). 2019
33. Khalid S., Rizwan G., Yasin H., Perveen R., Abrar H. Medicinal Importance of Holoptelea Integrifolia (Roxb). Planch–Its Biological and Pharmacological Activities. Nat Prod Chem Res 2: 124 doi: 10.4172/2329-6836.1000124 Page 2 of 4 seasonal variation of airborne pollen has been reported. Bulk of pollens originates from anemophilous trees and grasses. Thirteen pollen types have been recorded on more than 1% of annual total pollen catch. Holoptelea integrifolia. 2013
34. Nematbakhsh M., Ashrafi F., Safari T., Talebi A., Nasri H., Mortazavi M., et al. Administration of vitamin E and losartan as prophylaxes in cisplatin-induced nephrotoxicity model in rats. Journal of nephrology.**25**(3):410. 2012
35. Eshraghi-Jazi F., Nematbakhsh M., Nasri H., Talebi A., Haghighi M., Pezeshki Z., et al. The protective role of endogenous nitric oxide donor (L-arginine) in cisplatin-induced nephrotoxicity: Gender related differences in rat model. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences.**16**(11):1389. 2011

36. Ramhit P., Ragoo L., Bahorun T., Neergheen-Bhujun V. Multi-targeted effects of untapped resources from the Mauritian endemic flora. South African Journal of Botany.**115**:208-16. 2018
37. Morilla L.J.G., Demayo C.G. Medicinal Plants Used by Traditional Practitioners in Two Selected Villages of Ramon Magsaysay, Zamboanga Del Sur. Pharmacophore.**10**(1):84-92. 2019
38. Ayyanar M., Ignacimuthu S. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. J Ethnopharmacol.**134**(3):851-64. 2011
39. Shaheen S., Ramzan S., Khan F., Ahmad M. Adulteration A Global Issue. Adulteration in Herbal Drugs: A Burning Issue: Springer; 2019. p. 1-8.
40. Kumar D.P., Umasankar K., Alagusundaram M., Jayachandra Reddy P. Current Trends in Regulatory Authority Actions against Misbranded and Adulterated Drugs. International Journal of Advance Research, Ideas and Innovation in Technology.**3**(3):1513-21. 2017
41. Laloo D., Sahu A., Hemalatha S., Dubey S. Pharmacognostical and phytochemical evaluation of Cinnamomum wightii Meissn. flowers. 2012
42. Ghosh A., Laloo D., Singh N.K. Comparative estimation and chemical standardization of new and old sample of Chyawanprash. Int J Pharm Pharm Sci.**5**(3):801-4. 2013
43. Gautam A., Kashyap S.J., Sharma P.K., Garg V.K., Visht S., Kumar N. Identification, evaluation and standardization of herbal drugs: A review. Der Pharmacia Lettre.**2**(6):302-15. 2010
44. Kumar N., Chaudhary A. Pharmacognostic and phytochemical evaluation of Prunus persica (L.). International Journal of Research and Development in Pharmacy & Life Sciences.**6**(6):2806-12. 2017
45. Bhavana R., Thomas R.R., Binu. COMPARATIVE STUDIES ON MORPHOLOGY, ANATOMY AND PHYTOCHEMISTRY OF SELECTED SPECIES OF CROTON L.(EUPHORBIACEAE). Plant Archives.**20**(1):639-56. 2020
46. Dutta S., Chakraborty A.K., Dey P., Kar P., Guha P., Sen S., et al. Amelioration of CCl4 induced liver injury in swiss albino mice by antioxidant rich leaf extract of Croton bonplandianus Baill. PloS one.**13**(4). 2018
47. Somit D., Priyankar D., Kumar C.T. Quantification and correlation of the bioactive phytochemicals of Croton bonplandianum leaves of Sub-Himalayan region of West Bengal. Asian J Pharm Clin Res.**6**(3):142-7. 2013
48. Pande C., Tewari J. Chemical examination of Croton sparsiflorous. Journal Indian Chemical Society.**39**:545-52.1962
49. Qureshi S., Reddy A.V., Kumar G., Nousheen L. Pharmacognostic, physicochemical standardization and phytochemicalanalysis of leaves of Hydrolea zeylanica Vahl.(Hydrophyllaceae). Research Journal of Pharmacognosy and Phytochemistry.**9**(1):1-7. 2017
50. Dassanayaka G.G., Sivasinthujah S., Christy Jeyaseelan T. Physicochemical and preliminary phytochemical analysis of different brands ofHingwashtak churna; a polyherbal medicine in Sri Lanka. 2024
51. Organization W.H. Quality control methods for medicinal plant materials: World Health Organization; 1998.
52. Unit P., Organization W.H. Quality control methods for medicinal plant materials. World Health Organization, 1992.
53. Mohan V.R., Kalidass C. Nutritional and antinutritional evaluation of some unconventional wild edible plants. Tropical and subtropical Agroecosystems.**12**(3):495-506. 2010
54. Yumnamcha T., Nongthomba U., Devi M.D. Phytochemical screening and evaluation of genotoxicity and acute toxicity of aqueous extract of Croton tiglium L. International Journal of Scientific and Research Publications.**4**(1):1-5. 2014

55. Pucker B., Reiher F., Schilbert H.M. Automatic identification of players in the flavonoid biosynthesis with application on the biomedicinal plant Croton tiglium. Plants.**9**(9):1103. 2020
56. Shirmohammadli Y., Efhamisisi D., Pizzi A. Tannins as a sustainable raw material for green chemistry: A review. Industrial Crops and Products.**126**:316-32. 2018
57. Hassanpour S., MaheriSis N., Eshratkhah B. Plants and secondary metabolites (Tannins): A Review. 2011
58. Roy A. A review on the alkaloids an important therapeutic compound from plants. IJPB.**3**(2):1-9. 2017
59. Joosten L., van Veen J.A. Defensive properties of pyrrolizidine alkaloids against microorganisms. Phytochemistry Reviews.**10**(1):127-36. 2011
60. Gurrala R.C., Sahu S., Phani P.R., Prasad V., Babu B.K., Dobbidi P. An investigation of photocatalytic and biological properties of Croton bonplandianum-mediated Ag-Cu bimetallic nanoparticles. Journal of Photochemistry and Photobiology A: Chemistry.**454**:115729. 2024
61. Adebayo S.A., Masoko P. Therapeutic uses of plant species for inflammation-related conditions in Limpopo province of South Africa: a mini-review and current perspectives. Int J Pharmacog Phytoth Res.**1**(1):2-8. 2017
62. Uytan G., Tokgöz H.B., Ünal R., Altan F. Transcriptional analyses of the effects of Catharanthus roseus L. medicinal plant extracts on some markers related to obesity and inflammation in 3T3-L1 mouse cell lines. Biologia.**76**(1):297-306. 2021
63. Shewaiter M.A., Hammady T.M., El-Gindy A., Hammadi S.H., Gad S. Formulation and characterization of leflunomide/diclofenac sodium microemulsion base-gel for the transdermal treatment of inflammatory joint diseases. Journal of Drug Delivery Science and Technology.102110. 2020
64. Okokon J.E., Nwafor P.A. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of Croton zambesicus. Pak J Pharm Sci.**23**(4):385-92. 2010
65. Adegbola P., Aderibigbe I., Hammed W., Omotayo T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. Am J Cardiovasc Dis.**7**(2):19. 2017
66. Handique J., Baruah J. Polyphenolic compounds: an overview. Reactive and Functional Polymers.**52**(3):163-88. 2002
67. Arslan R., Bektas N., Ozturk Y. Antinociceptive activity of methanol extract of fruits of Capparis ovata in mice. J Ethnopharmacol.**131**(1):28-32. 2010
68. Lessa T.L.A.d.S., Correia T.M.L., Santos T.C.d., da Silva R.P., Silva B.P.d., Cavallini M.C.M., et al. A novel diselenide attenuates the carrageenan-induced inflammation by reducing neutrophil infiltration and the resulting tissue damage in mice. Free Radic Res.**58**(4):229-48. 2024