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Antioxidant and Anti-Inflammatory Studies of Leaf of Ricinus Communis and Rhizome of Curcuma Amada for Topical Application

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Abstract:- Medicinal plants have been used in Ayurveda for treatment of topical inflammation, skin wound owing to their antioxidant and anti-inflammatory activities. The present study deals with the comparative evaluation of invitro antioxidant and anti-inflammatory potential of aqueous, ethanolic and methanolic extracts of leaf and rhizome of *Ricinus communis* and *Circuma amada* respectively. The aqueous and alcoholic extracts were prepared by homogenization. Their thin layer chromatography profiles were studied for the presence of bioactive phytochemicals. The qualitative phytochemical screening was carried out by standard biochemical assays. Total phenolic and flavonoid contents of extracts were determined by colorimetric estimation to find their correlation with antioxidant activity. Invitro anti-oxidant activity was evaluated by ferric reducing power assay and total antioxidant capacity. Invitro anti-inflammatory activity of leaf and rhizome extracts was examined by protease inhibition activity, albumin denaturation inhibition assay and membrane stabilization.TLC profiling of extracts of both plants showed presence of phytochemicals with Rf values between 0.09-1 for *R. communis* and 0.12-0.57 for *C. amada.* The highest total phenolic (12.8±0.253mg GAE/gm extract) content was observed in methanolic extract of *R. communis* and flavonoid (303±0.157mg QE/gm extract) content in ethanolic extract of *C. amada*. Results Zdemonstrated that the alcoholic extract of *R. communis* showed significant anti-inflammatory activity by inhibiting heat induced albumin denaturation, 60.63±0.23% haemolysis inhibition, and antiprotease activity as compared to *C. amada*. The study revealed that alcoholic extracts of *R. communis* contains potent antioxidant compounds, which possesss anti-inflammatory activity, justifying its popular uses in traditional medicine.

Keywords:- *Ricinus communis; Curcuma amada; inflammation; antioxidant; invitro.*

I. INTRODUCTION

Ancient cultures across the globe, dating long before prehistoric times, have used plants in various forms for their medicinal benefits. In the Indian scriptures of Ayurveda, a lot many plants are mentioned for their various dietary and medicinal uses. Amongst the many plant benefits mentioned, antioxidant and anti-inflammatory properties are of prime importance and have gained much interest from the scientific community recently.

Antioxidants act against free radicals and reactive oxygen species (ROS) which are unstable molecules that cause slow damage to the cell's structure and function. Antioxidants like flavonoids, alkaloids, and polyphenols exhibit anti-inflammatory, anti-carcinogenic, and antibiotic activities alongside having other physiological and pharmacological effects [1]. Anti-inflammatory agents reduce inflammation both on the surface and internal levels of the body. Inflammation in the body is elicited in response to infection, irritation, or injury [2]. *A. sativum* (garlic) [3,4], *Camellia sinensis* (green tea) [5,6,7] *Curcuma longa* (turmeric) [8], *Emblica officinalis* (Indian gooseberry), *Zingiber officinalis* (ginger) [9] etc. are some of the plants used for both dietary and medicinal purposes in Ayurveda owing to their antioxidant effects and anti-inflammatory properties [10].

Curcuma amada, commonly known as amba haldi or mango ginger, is closely related to turmeric, *Curcuma longa*. Both belong to the family Zingiberaceae. Amba haldi has been a part of the Indian Ayurveda for its properties that bring relief to joint pain pain [11], skin problems, indigestion, cough, and healing of wounds. Its rhizome looks very similar to ginger. It is used in Thai, Indian and other cuisines of Asian origin for its raw mango-like taste, and spicy flavor. It has also been used for healing of wounds, cut and itching [12].

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Curcumin (diferuloylmethane) is a bright yellow-colored phenolic compound found in turmeric. The phytochemical has been proven to have various dermatological benefits [13] alongside an array of pharmacological properties like anti-inflammatory, antimicrobial [14] antioxidant [15], and antitumor properties [16, 17]. Many additional encouraging effects have been observed in other pro-inflammatory diseases like arthritis, irritable bowel disease, gastric inflammation and ulcers, diabetic nephropathy and microangiopathy [18, 19, 20].

Ricinus communis commonly called the Castor oil plant or castor bean belongs to the spurge family Euphorbiaceae. Chiefly cultivated in India, Brazil, and China, the plant is believed to be native to tropical Africa. Castor leaves and roots are used in ayurvedic treatments. In Ayurveda, an emphasis is made on Eranda (*Ricinus Communis Linn*) root for its anti-inflammatory and analgesic properties [21].

When a study of phytochemical and pharmacological activities was done on leaf of *Ricinus communis* plants, it exhibited a plethora of anti-inflammatory, antimicrobial, antioxidant, insecticidal, and wound-healing activities [22]. The leaves are being used to treat inflammation manifestations in north India along with turmeric paste [23]. The phytochemicals present have free radical scavenging, Hg scavenging, and repelling activities. The benefits of the plant are also extended to the treatment of ulcers, gastric inflammations, constipation, warts, headaches, etc [24, 25].

Till now, there are no reports available on comparative anti-inflammatory activity of castor leaves and rhizome of *C. amada* for topical application. Keeping this in view, the present study was conducted to evaluate comparative invitro phytochemical analysis, antioxidant activity and anti-inflammatory effects of rhizome of *C. amada* and leaves of *R. communis*.

II. MATERIALS AND METHODS

A. Collection of plant materials

The leaves of castor plant [Fig-1] were collected from a medicinal plant nursery and rhizome of *Curcuma amada* was purchased from the local vegetable market.

Fig. 1: Leaves of Ricinus communis and rhizome of Curcuma amada

B. Plant extract preparation

The rhizome [Fig-2] of *Curcuma amada* and castor leaves were washed, air dried. Rhizome was peeled and chopped into smaller pieces. Castor leaves and rhizome were grinded in suitable solvent with the help of mortar and pestle. The extracts of the plants were prepared by soaking 6.5 gms of finely ground rhizome and leaves in 15ml of water, ethanolic and methanolic solvents separately for 12 hours. The extracts were then filtered using Whatman filter paper No.42. Collected filtrates were used for carrying out different biochemical assays.

Fig. 2: Peeled rhizome of *Curcuma amda*

C. Thin Layer Chromatography

Thin layer chromatography was carried out on silica gel G (400 mesh size) plates made manually in laboratory. The samples were loaded 2 cm above from the bottom of the plates with the help of micropipettes to uniformly apply the samples and allowed to dry. The plates were developed in a chromatography chamber using solvent system as chloroform: methanol (15: 1). The plates were air dried and then kept in hot air oven at 100 °C for 5-6 minutes and then were observed and visualized under visible light. The retention factor (R_f values) for each active compound was calculated for visible light.

D. Qualitative Phytochemical Screening

The different qualitative chemical tests were performed to detect various phytochemicals present in rhizome of amba haldi and leaf extract of castor plant [26, 27, 28]. The tests were performed as follows (Table 1):

a) Test for flavonoids
Alkaline reagent test: 2 ml of different extracts was mixed with 2 ml of 10% sodium hydroxide solution. An intense yellow colour was formed which turned colourless after addition of few drops of dilute acid indicated the presence of flavonoids.

b) Test for phenols
Ferric chloride test: 1-2 ml of different extract was treated with 1 ml of 5% ferric chloride solution. Appearance of blue black colour indicates the presence of phenolic compounds.

c) Test for terpenoids
Salkowski Test: 2 ml of each of the extract was treated with 1 ml of chloroform. Concentrated H_2SO_4 was carefully added along the side of the test tube to form a layer. A reddish-brown colouration at the interface indicates the presence of terpenoids.

d) Test for glycosides
Keller-Kellani test: 5 ml of different extracts was treated with 2 ml glacial acetic acid and 1 ml of 5% ferric chloride. After gentle heating transfer it to a test tube containing 2 ml of conc. H_2SO4. Appearance of reddish-brown colour at junction of two liquid and bluish green colour of acetic acid layer indicates the presence of glycosides.

e) Test for tannins
Braymer's test: 1 ml of different extracts were treated with 2 ml of 5% ferric chloride solution. Appearance of blue-black colour indicates the presence of tannins.

f) Test for steroids
Salkowski test: 1 ml of different extracts was treated with 1 ml of chloroform and concentrated sulphuric acid was added along the side of the test tube and shaken well. Chloroform layer appears red and acid layer showed greenish yellow colour.

g) Test for saponins
Foam Test: 2 ml of extract was diluted with 5 ml distilled water in a test tube and it was shaken vigorously. Formation of stable foam was taken as an indication for the presence of saponin.

S.No.	Metabolite	Test	Experiment	Observation
1.	Flavonoids	Alkaline reagent Test	2-3 ml extract + 2 ml 40% NaOH	Deep yellow colour appears
2.	Terpenoids	Salkowsky's Test	2 ml extract + 1 ml chloroform + few drops of conc, H_2SO_4	Reddish-brown colouration appears at the interface
3.	Glycosides	Kellarkialliani Test	5 ml extract + 2 ml glacial acetic acid + 1 ml 5% $FeCl_3$ + heat carefully then cool +transfer it to a TT containing 2 ml conc. H_2SO_4	Reddish- brown and greenish- blue ring appears at the junctions
4.	Saponins	Foam Test	2 ml extract + 5 ml D/W + shake TT	Stable foam
5.	Tannins	Braymer's Test	1 ml extract + 2 ml of 10% $FeCl_3$	Dark blue colour appears
6.	Phenols	Ferric chloride Test	1-2 ml extract + 1 ml of 5 % $FeCl_3$	Deep blue colour appears
7.	Steroids	Salkowsky's Test	1 ml extract + 1 ml chloroform +1 ml conc. H_2SO_4 along the sides of test tube	Chloroform layer appears red and acid layer shows greenish yellow colour.

Table 1: Qualitative analysis of phytochemicals

E. Total Phenolic Content (TPC)

Total phenol content was determined using the Folin-Ciocalteu reagent [29]. To 100 µl of extract, 1 ml distilled water, 5 ml of Folin- Ciocalteu reagent (10%) and 4 ml of 1M sodium carbonate were added and mixed properly. The absorbance was read after 30 min incubation at room temperature at 750 nm spectrophotometrically against a blank. A calibration curve of gallic acid was generated at 750 nm for concentrations ranging from 25µg/ml to 200µg/ml. The concentration of phenolics in the test samples was calculated from the calibration plot. Total phenolic content was expressed as ug of gallic acid equivalents (GAE)/gm of extract. All measurements were done in triplicates.

F. Total Flavonoid Content (TFC)

The standard Aluminium Chloride method was used to determine the total flavonoid content (TFC) in aqueous and alcoholic extracts of plants [30]. An aliquot of 0.1ml of test sample was mixed with 1ml of distilled water, 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate solution (1M). In the mixture, 2 ml of distilled water was added to bring up the total volume to 3.3 ml. The test solution was shaken vigorously and absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin ranging from100 µg/ml to 500 µg/ml against blank. The concentration of flavonoid in the test samples was calculated from the calibration plot.

G. Ferric Reducing Power Assay (FRPA)

Ferric ion reducing power was measured by colorimetric method [31]. Reducing power is a reflection antioxidant activity of any compound.0.1ml of stem extracts were mixed with 1ml sodium phoshphate buffer (0.2 M; pH=6.6) and 1 ml 1% potassium ferricyanide and incubated at 50 °C for 20 minutes. After that, 1ml of 10% TCA was added to the mixture and centrifuged at 3000r.p.m. for 10 minutes. 1.5 ml supernatant was then added to 1.5ml of distilled water and 0.1 ml of 0.1% ferric chloride ($FeCl_3$). The mixture was left aside at room temperature for 10 minutes and then O.D. was recorded at 700nm using spectrophotometer. Ascorbic acid (1mg/ml) was used as standard in concentrations of 25-125 ug/ml. Higher reducing power is indicated by high absorbance. All assays were run in triplicates.

H. Total Antioxidant Capacity (TAC)

This assay is based on the reduction of phosphate-Mo (VI) to Phosphate-Mo (V) by the sample and subsequent formation of a bluish green colored phosphate-Mo (V) complex at acidic pH [32].The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. An aliquot of 0.1 ml of sample solution was mixed with 1.2 ml distilled water and 2.2 ml phoshphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. After cooling, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml

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of distilled water in place of plant sample and incubated under the same conditions. Ascorbic acid was used as standard in concentrations of 25-125 ug /ml. The antioxidant capacity was reported as mg of ascorbic acid equivalents (AAE) per gram of extract.

I. Assessment of In-vitro Anti-inflammatory Activity

a) Inhibition of albumin denaturation

The anti-inflammatory activity of *R. communis* and C. *amada* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al. [33] and Chandra et al. [34] followed with minor modifications. The reaction mixture consisted of test extracts (0.2 and 0.4 m), 0.4 ml of 1% aqueous solution of bovine albumin fraction, and 2.8 ml PBS, pH 6.4. As positive control, distilled water (0.2ml) was used instead of extracts while 0.4 ml aspirin (150mg/10ml) was used as reference anti-inflammatory drug. The samples were incubated at 37 ºC for 5 min and then heated to 70 º C for 15 min, after cooling the samples, the turbidity was measured at 660nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

b) Antiproteinase action

The test was performed according to the modified method of Oyedepo et al. [35] with some modifications. The reaction mixture consisted of 100ul trypsin (10ug/ml), 0.5 ml 20 mM Tris HCl buffer (pH 7.4) and 0.5 ml test sample. The mixture was incubated at 37°C for 5 min and then 0.5 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 1 ml of 10% trichloroacetic acid was added to arrest the reaction. As control, distilled water (0.5ml) was used instead of extracts while 0.5 ml aspirin (150mg/10ml) was used as reference anti-inflammatory drug. Cloudy suspension was centrifuged and the anti proteinase activity of extracts was confirmed by Biuret test. 1ml of Biuret reagent (0.5% $CuSO_4$ and 10% NaOH) was added to the supernatant obtained after centrifugation. The extract showing deep blue or violet colour when compared to control confirmed its anti proteinase action.

c) Membrane stabilization

The study was performed according to Sakat et al. [36], Sadique et al. [37] and Shinde et al. [38] with minor modifications as follows:

a. Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood was collected from pathology and transferred to the heparinised centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

b. Heat induced haemolysis

The reaction mixture consisted of 20ul test extract, 100ul of 10% RBCs suspension and 3.0 ml 10 mM PBS, pH 7.4. 20 ul of saline was added to the control test tube instead of test extract. Aspirin (150mg/10ml) was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

III. STATISTICAL ANALYSIS

All the experiments were carried out in triplicate and the results were given as the mean ± standard deviation (SD). The data were analyzed for statistical significance using Student's *t*-test and differences were considered significant at $p < 0.05$.

IV. RESULTS

A. Percentage yield and color of plant extract

The percentage yield and color [Fig-3] of each extract is presented in Table 2.

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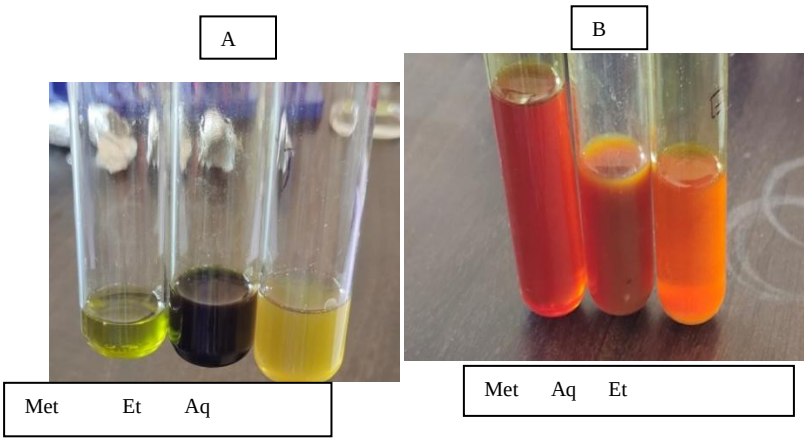

Fig. 3: Methanolic, ethanolic and aqueous extract of (A): *R.communis and (B): C. amada*

Plant Extract	Percentage Yield	Color
Castor Ethanolic	58.6	Dark olive green
Castor Methanolic	53.3	Ligtr olive green
Castor Aqueous	70	Pale green
Amba Ethanolic	44	Bright orange
Amba Methanolic	69.3	Dark orange
Amba Aqueous	86.6	Dark brown

Table 2: Percentage yield and color of extracts

B. Thin Layer Chromatographic Studies

TLC profiling of ethanolic and methanolic and aqueous extracts of amba haldi and castor plants gave an idea about the presence of various coloured phytochemicals. Different R_f values of phytochemicals showed their polarity and enabled selection of suitable solvent for their isolation. The presence of coloured spots observed under visible light has been shown in figure [Fig-4] and comparative analysis is presented in Table 3. The different R_f values of alcoholic and aqueous extracts of both plants indicated that phytochemicals extracted all the extracts are alike and are important in antioxidant activity of this plant.

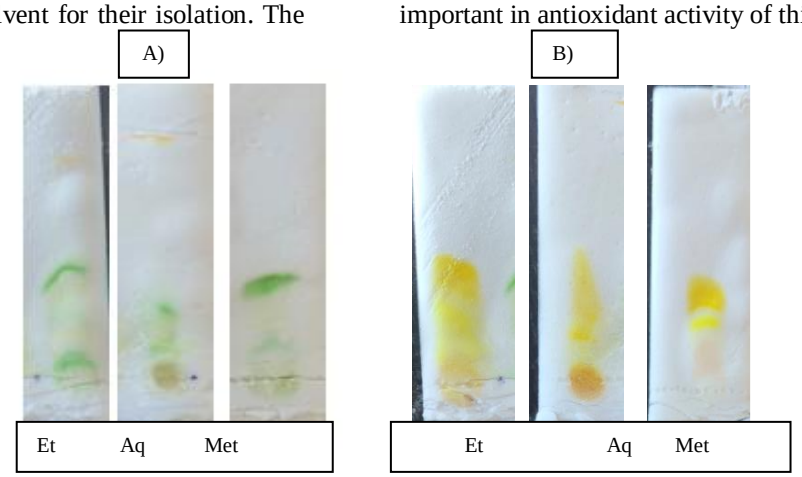

Fig. 4: TLC profile of ethanolic, water and methanoliextract of (A): *R. communis* and (B): *C. amada*

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S.No.	Fraction	No. of spots	Colour	R_f
1	Castor Ethanolic	4	Green Yellow Green Orange	0.12 0.44 0.51 1
2	Castor Aqueous	4	Green Yellow Green Orange	0.14 0.26 0.31 1
3	Castor Methanol	4	Dark Green Yellow Green Orange	0.09 0.30 0.38 1
4	Amba Ethanol	3	FluorescentBrown Yellow Dark Yellow	0.14 0.36 0.57
5	Amba Aqueous	3	FluorescentBrown Yellow Dark Yellow	0.12 0.37 0.47
6	Amba Methanol	3	FluorescentBrown Yellow Dark Yellow	0.20 0.32 0.48

Table 3: TLC of extracts of castor and amba haldi

C. Phytochemical Screening

Phytochemicals like flavonoids and polyphenols are the most important groups of secondary metabolites in plants which possess diverse biological activities like antioxidant and anti-inflammatory properties. The phytochemical analysis of ethanolic, methanolic and aqueous extracts of *R.communis and C.amada* demonstrated presence of various secondary phytochemicals like flavonoids, terpenoids, glycosides, steroids (Table 4).The colored reactions for each chemical test are presented in figures below [Fig-5-Fig-8].

Phytochemicals like polyphenols, flavonoids, terpenoids, glycosides and steroids are present in ethanolic, methanolic and aqueous extracts of *R. communis and C. amada*. Saponin was absent in alcoholic and aqueous extract of *C. amada and* methanolic extract of *R. communis.* Ethanolic extracts contain good amount of flavonoids and saponins as compared to methanolic and aqueous extracts of both plants. These phytochemicals were better extracted in ethanol than water and methanol which may contribute to its high antioxidant activity. These results suggested that phytochemicals like flavonoids, polyphenols, tannins, terpenoids, glycosides and steroids are the active constituents in *R. communis* and *C. amada.* The result of this study is agreed with the previous study reported by Sutar et al. [39] and Ribeiro et al. [40].

Sr. no	Plants	*R. communis*			*C. amada*		
	EXTRACT	Ethanolic	Methanolic	Aqueous	Ethanolic	Methanolic	Aqueous
1.	Flavonoids	++	+	+	++	+	+
2.	Phenols	++	++	++	++	++	++
3.	Terpenoids	++	++	+	++	++	+
4.	Glycosides	++	++	+	++	++	+
5.	Saponins	++	-	+	-	-	-
6.	Tannins	++	++	++	++	++	++
7.	Steroids	++	++	+	++	++	+

Table 4: Qualitative phytochemical analysis of *R.communis* and *C. amada*

Present + Strongly present ++ Absent –

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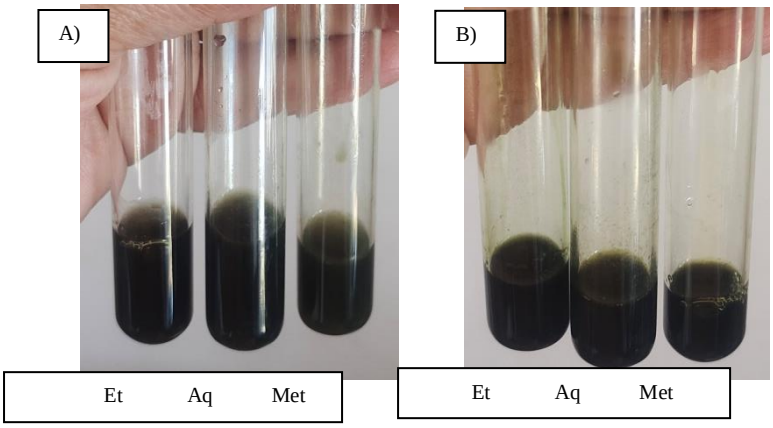

Fig. 5: Polyphenols in ethanolic, methanolic extracts of (A): *R. communis* and (B): *C. amada*

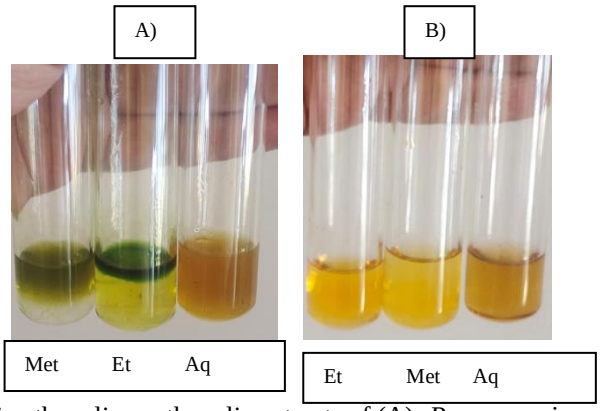

Fig. 6: Flavonoid in ethanolic, methanolic extracts of (A): *R. communis* and (B): *C. amada*

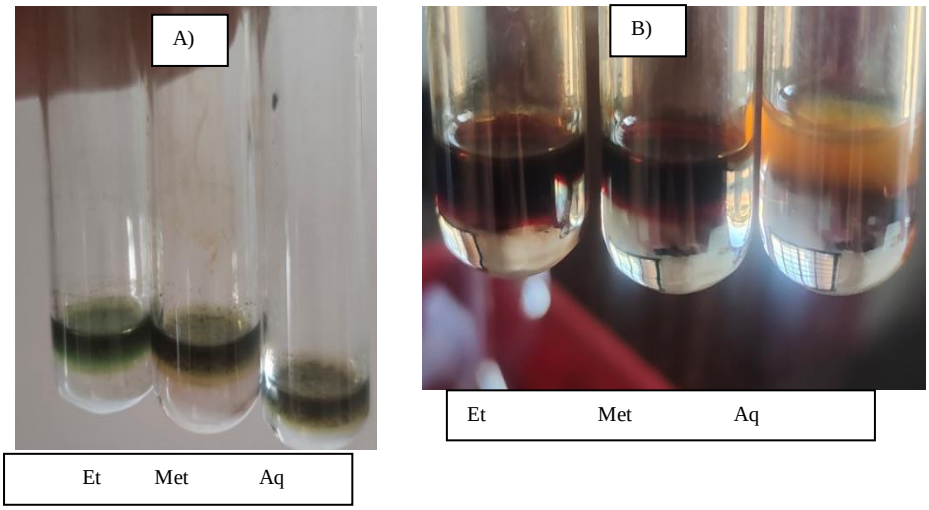

Fig. 7: Glycosides in ethanolic, methanolic extracts of (A): *R. communis* and (B): *C. amada*

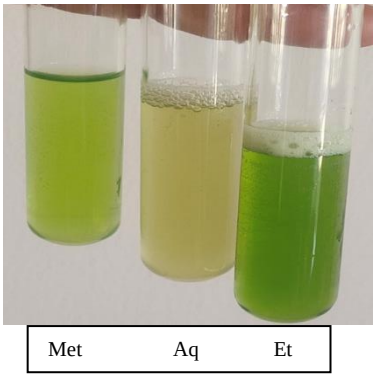

Fig. 8: Saponin in ethanolic, methanolic extracts of *R. communis*

D. Total Phenolic Content (TPC)

The total phenolic content of alcoholic and aqueous extracts of *R .communis and C. amada* was estimated using gallic acid as standard from the calibration curve [Fig-9]. They were 12.8± 0.253, 8.3±0.168 and 7.8±0.211 mg gallic acid equivalents (GAE)/gm of sample in methanolic, ethanolic and aqueous extracts of *R. communis*. The ethanolic, methanolic and aqueous extracts of C. amada contained 4.8± 0.183, 4.72± 0.246 and 1.63± 0.313 mg gallic acid equivalents (GAE)/gm of sample. The phenolic content in *R. communis* was found to be higher than that of *C. amada*. The highest phenolic content was observed in methanolic extract of *R. communis* and minimum in aqueous extract of *C. amada*. The experimental results obtained from the extracts are presented in Table 5.

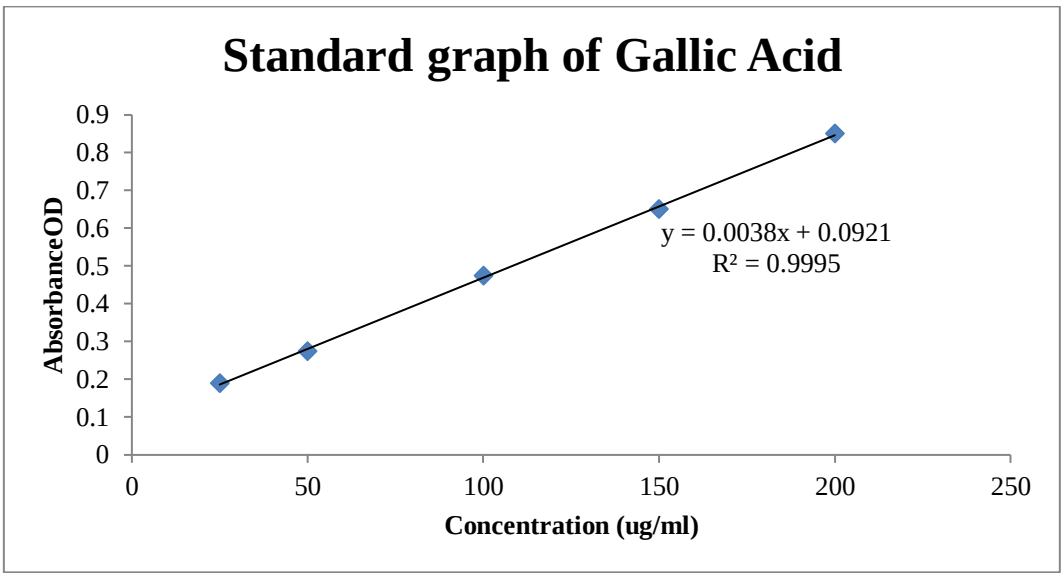

Fig. 9: Standard curve of Gallic acid

E. Total Flavonoid Content (TFC)

Total flavonoid contents were extrapolated from the straight line equation of quercetin standard curve [Fig-10]. TFC obtained for ethanolic, methanolic and aqueous extracts of *R. communis* was 145±0.311 and 83.5±0.221 and 42±0.141 mg quercetin equivalent (QE)/g extract respectively (Table 5). The content of flavonoids in ethanolic, methanolic and aqueous extracts of *C amada* was 303±0.157, 233±0.251, 67±0.431 mg quercetin equivalent (QE)/g extract. The total flavonoid content in alcoholic extracts of *C. amada* was significantly higher than extracts of *R. communis*.

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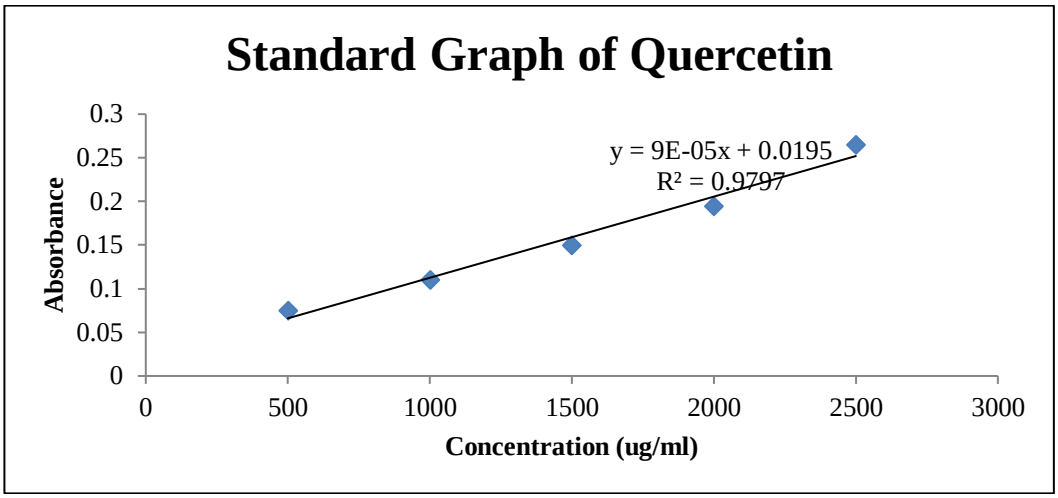

Fig. 10: Standard curve of Quercetin

F. Ferric Reducing Potential Assay (FRPA)

This assay measures reducing potency of plant extract against the oxidative effects of reactive oxygen species. It is based on the reduction of ferric ion via the addition of hydrogen removed from phenolic antioxidant compound. The higher absorbance indicates higher reducing potency of the sample. The mean values of FRPA presented in Table 5. The methanolic extract of *R.communis* revealed maximum antioxidant potential as 3.98±0.157mg ascorbic acid equivalent (AAE)/gm extract. Lower FRPA value was observed for aqueous extract of *C. amada* as 900±0.300ug AAE/gm. Ascorbic acid at the concentration of 1mg/ml was used as the reference antioxidant for comparison.

G. Total Antioxidant Capacity (TAC)

This method evaluates both water soluble and fat soluble antioxidants. The results indicate higher TAC of the ethanolic extract of Hadjod plant as compared to their methanolic extract. The mean values regarding the antioxidant potential capacity of both the extracts of plant are shown in Table 5. Methanolic extract of *R.communis* demonstrated the highest antioxidant potential (330.7±0.757 ug AAE/gm) and the lowest were in aqueous extract of *C. amada* (0.416±0.132mg AAE/gm).Ascorbic acid at the concentration of 1mg/ml was used as the reference antioxidant for comparison.

Plant	Extract	TPC (mgGAE/gm extract)	TFC (mgQE/gm extract)	FRPA (mgAAE/gm extract)	TAC (mgAAE/gm extract)
R. communis	Ethanolic	8.3±0.168	145±0.311	2.57±0.234	1.08±0.347
	Methanolic	12.8±0.253	83.5±0.221	3.98±0.157	2.25±0.294
	Aqueous	7.8±0.211	42±0.141	1.9±0.371	1.916±0.186
C. amada	Ethanolic	4.8±0.183	303±0.157	2.21±0.422	1.583±0.286
	Methanolic	4.72±0.246	233±0.251	1.98±0.165	1.75±0.362
	Aqueous	1.63±0.313	67±0.431	0.9±0.300	0.416±0.132

Table 5: Quantitative phytochemical and antioxidant assay of R. *communis* and *C. amada*

Values are expressed as mean ±SD for three determinations

H. Anti-Inflammatory Studies

a) Inhibition of albumin denaturation

Denaturation of protein is the major cause of inflammation. The ability of ethanolic, methanolic and aqueous extracts of *R. communis* and *C. amada* to inhibit protein denaturation was studied for their anti-inflammatory action. The ethanolic extract was significantly effective in inhibiting heat induced albumin denaturation as compared to methanolic extract. Maximum inhibition of 52.6±0.55 % was observed for 50 ul of ethanolic extract (433mg/ml) of *R. communis* while for similar volume, aqueous extract of *C. amada* showed least (19.9±0.462%) inhibition. Aspirin was used as a standard anti-inflammatory drug and it showed 42.7±0.63% inhibition for 50 ul as compared to control. Results are presented in Table 6.

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Plant	Sample	Concentration (mg/ml)	Volume (ul)	Albumin denaturation (% inhibition)
R. communis	Ethanolic	433	50	52.6±0.55
	Methanolic	433	50	39.05±0.54
	Aqueous	433	50	49.1±0.43
C. amada	Ethanolic	433	50	36.8±0.51
	Methanolic	433	50	26.3±0.25
	Aqueous	433	50	19.9±0.462
	Aspirin	15	50	42.7±0.63

Table 6: Effect of extract of *R. communis* and *C. amada* on albumin denaturation

Values are expressed as mean ±SD for three determinations

b) Proteinase inhibitory activity

The ethanolic stem extract of *R. communis* and *C. amada* demonstrated significant antiproteinase activity as compared to methanolic extract. Neutrophil proteinases play important role in the development of tissue damage during inflammation and significant protection can be provided by any agent which can inhibit proteinase activity thus acting as anti-inflammatory. Maximum inhibition by ethanolic extract of both plants was inferred due to dark purple/blue appearance after application of Biuret test which indicates inhibition of protease action on casein as compared to control (CTL) [Fig-11]. Methanolic extract of *R. communis* was also equivalent to ethanolic extract in its antiproteinase activity. Control showed light blue/purple colour due to action of protease on casein. Aqueous extracts of *R. communis and C. amada* showed light blue/purple colour upon Biuret test which revealed that it is unable to inhibit protease activity. Aspirin as standard was also able to inhibit protease activity as demonstrated by its dark blue colour (lesser than ethanolic stem extract) after Biuret test.

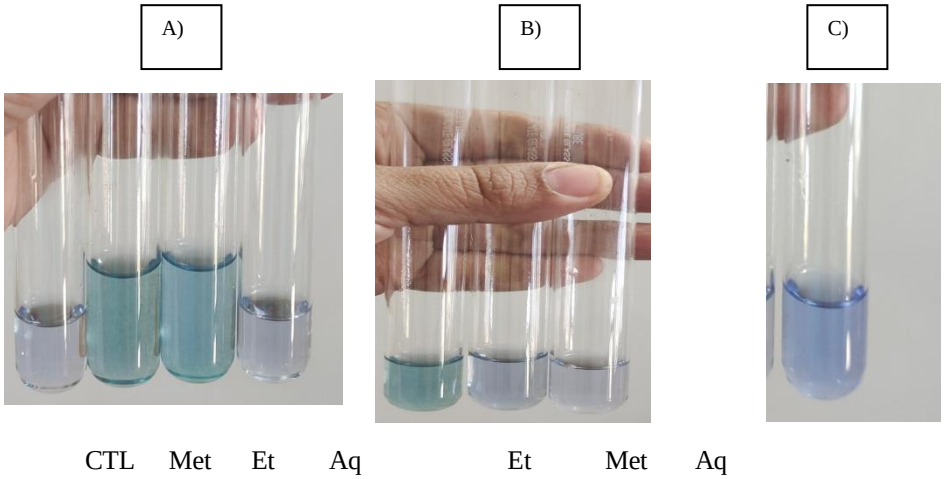

Fig. 11: Antiproteinase activity of extract of (A): *R. communis*; (B): *C. amada* and (C): Aspirin

c) Membrane stabilization

Stabilization of RBCs membrane was studied to establish an additional mechanism for anti-inflammatory action of ethanolic, methanolic and aqueous extracts of amba haldi and castor plant.. Due to resemblance of RBC membrane with lysosomal membrane, this effect may possibly inhibit the release of lysosomal content of neutrophils responsible for tissue damage at the site of inflammation. The ethanolic extract of *R. communis* significantly inhibited heat induced hemolysis of RBCs with maximum inhibition 60.63±0.23 % at 433mg/ml. Aqueous *C. amada* extract showed 10.23±0.29% hemolysis inhibition which was least. Aspirin, standard drug showed inhibition 48.37±0.52 at 15mg/ml (Table 7). As seen in figure, [Fig-12] inhibition of hemolysis is inferred in ethanolic extract of *R. communis* by light red colouration as compared to control (CTL).

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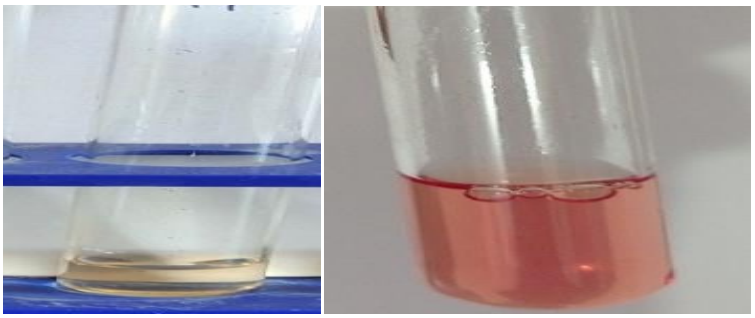

Et **CTL**

Fig. 12: Hemolysis inhibition by ethanolic extract of *R. communis*

Plant	Sample	Concentration (mg/ml)	Volume(ul)	% hemolysis inhibition
R. communis	Methanolic	433	5	60.63±0.23
	Ethanolic	433	5	43.76±0.43
	Aqueous	433	5	55.32±0.58
C. amada	Ethanolic	433	5	19.89±0.18
	Methanolic	433	5	18.79±0.35
	Aqueous	433	5	10.23±0.29
	Aspirin	15	20	48.37±0.52

Table 7: Effect of *R. communis* and *C. amada* on membrane stabilization

Values are expressed as mean ±SD for three determinations

V. DISCUSSION

The present study demonstrated the evidence for the antioxidant and anti-inflammatory effect of *R. communis* and *C. amada* and can prove to be effective anti-inflammatory drugs for topical inflammation. Treatment of wound healing in rabbits with castor leaf extract had been earlier reported by Mohammed and Albozachri [41]. Sharma et al. [42] demonstrated wound healing in rats using 10% ethanolic extract of C. amada rhizomes. Antioxidant potential of *R. communis* and *C. amada* is clearly revealed in FRPA and TAC assay. The inhibition of albumin protein denaturation, antiproteinase action and membrane stabilization was studied to establish the mechansim of anti-inflammatory activity of both the plants. The results of these mechanisms showed greater potential of alcoholic extract of *R. communis* and *C. amada* to be used as anti-inflammatory agent. Thus, alcoholic extract has the greatest ability to extract potential antioxidant metabolites.Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activity of many plants [43]. Hence anti-inflammatory activity of *R. communis* and *C. amada* might be due to the presence of significant amount of secondary metabolites such as polyphenols and flavonoids.The similar results were obtained for demonstration of antioxidant property of *C. amada* by Hait and Deepak [44]. Saini et al. [45] demonstrated anti-inflammtory activity of *R. communis* mainly due to flavonoids.

This study revealed that both methanolic and ethanolic leaf extract of *R. communis* proved to be a better anti-inflammatory agent compared to *C.amada* and showed comparable effect to Aspirin. The alcoholic extracts of *C. amada* though rich in phytochemicals and potent in antioxidant activity but was less effective in producing anti-inflammatory response than *R. communis*.These findings justify the use of *R. communis* in traditional medicine in management of topical inflammation but further investigations in terms of profiling of phytochemical content in *R. communis* using HPTLC/HPLC is essential to figure out the active candidate or chemical entity that is mainly responsible for this activity. More studies are required to validate its anti-inflammatory effect in clinical settings in the current paradigm of inflammation.

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ETHICAL STATEMENT

The experimental protocols were approved by the Institutional Ethical Committee of IILR (IEC No: 19/07/21) Academy, Indore.

REFERENCES

[1.] Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Crit Rev Food Sci Nutr 2018; 8; 1260-1270.

[2.] Bayan, L.; Koulivand, P.H.; Gorji, A. Garlic: a review of potential therapeutic effects. Avicenna J Phytomed 2014; 4(1);1-14.

[3.] Afzal, M.; Safer, A.M.; Menon, M. Green tea polyphenols and their potential role in health and disease. Inflammopharmacology 2015; 23(4); 151-61.

[4.] Arreola, R.; Quintero-Fabián, S.; López-Roa, R.I.; Flores-Gutiérrez, E.O.; Reyes-Grajeda, J.P.; Carrera-Quintanar, L.; Ortuño-Sahagún, D.

Immunomodulation and anti-inflammatory effects of garlic compounds. J Immunol Res 2015; 401630.
[5.] Oz, H.S. Chronic inflammatory diseases and green tea polyphenols. Nutrients 2017; 9(6): 561.
[6.] Ohishi, T.; Goto, S.; Monira, P.; Isemura, M.; Nakamura, Y. Anti-inflammatory action of green tea. Antiinflamm Antiallergy Agents Med. Chem 2016; 15(2); 74-90.
[7.] Kuptniratsaikul, V., Thanakhumtorn, S.; Chinswangwatanakul, P.; Wattanamongkonsil, L.; Thamlikitkul, V. Efficacy and safety of Curcuma domestica extracts in patients with knee osteoarthritis. J Altern. Complement Med 2009; 15(8):891-7.
[8.] Tabrizi, R.; Vakili, S.; Akbari, M.; Mirhosseini, N.; Lankarani, K.B.; Rahimi, M.; Mobini, M.; Jafarnejad, S.; Vahedpoor, Z.; Asemi, Z. The effects of curcumin-containing supplements on biomarkers of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials. Phytother. Res 2019; 33(2); 253-262.
[9.] Morvaridzadeh, M.; Fazelian, S.; Agah, S.; Khazdouz, M.; Rahimlou, M.; Agh, F.; Potter, E.; Heshmati, S.; Heshmati, J. Effect of ginger (*Zingiber officinale*) on inflammatory markers: A systematic review and meta-analysis of randomized controlled trials. Cytokine 2020; 135; 155224.
[10.] Shukla, S.D.; Bhatnagar, M.; Khurana, S. Critical evaluation of ayurvedic plants for stimulating intrinsic antioxidant response. Frontiers in Neuroscience 2012; 6(112);1-12
[11.] Daily, J.W.; Yang, M.; Park, S. Efficacy of turmeric extracts and curcumin for alleviating the symptoms of joint arthritis: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J Med Food 2016; 219(8); 717-29.
[12.] Srivastava, A.K.; Srivastava, S.K.; Shah, N.C. Constituents of the rhizome essential oil of *Curcuma amada* Roxb. from India. J Essent. Oil Res 2001; 13(1); 63-64.
[13.] Vaughn, A.R.; Branum, A.; Sivamani, R. K. Effects of turmeric (*Curcuma longa*) on skin health: A systematic review of the clinical evidence. Phytother Res 2016; 30(8); 1243-64.
[14.] Gupta, S.C.; Patchva, S.; Aggarwal, B.B. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J 2013; 15(1); 195-218.
[15.] Barua, N.; Buragohain, A.K. Therapeutic potential of curcumin as an antimycobacterial agent. Biomolecules 2021; 11(9); 1278.
[16.] Aggarwal, B.B.; Kumar, A.; Bharti, A.C. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003; 23(1A); 363-98.
[17.] Vera-Ramirez, L.; Pérez-Lopez, P.; Varela-Lopez, A.; Ramirez-Tortosa, M.; Battino, M.; Quiles, J.L. Curcumin and liver disease. Biofactors 2013; 39(1); 88-100.
[18.] Aggarwal, B.B.; Harikumar, K.B. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. J Biochem Cell Biol 2009; 41(1); 40-59.
[19.] Kuptniratsaikul, V.; Dajpratham, P.; Taechaarpornkul, W.; Buntragulpoontawee, M.; Lukkanapichonchut, P.; Chootip, C.; Saengsuwan, J.; Tantayakom, K.; Laongpech, S. Efficacy and safety of *Curcuma domestica* extracts compared with ibuprofen in patients with knee osteoarthritis: a multicenter study. Clin Interv Aging 2014; 20(9); 451-8.
[20.] Wright, L.E.; Frye, J.B.; Gorti, B.; Timmermann, B.N.; Funk, J.L. Bioactivity of turmeric-derived curcuminoids and related metabolites in breast cancer. Curr Pharm Des 2013; 19(34); 6218-25.
[21.] Elkousy, R.H.; Said, Z.N.A.; Abd El-Baseer, M.A.; Abu El Wafa, S.A. Antiviral activity of castor oil plant (Ricinus communis) leaf extracts. J Ethnopharmacol 2021; 10(271); 113878.
[22.] Suurbaar, J.; Mosobil, R.; Donkor, A.M. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of Ricinus communis against selected pathogens. BMC Res Notes 2017; 10(1); 660.
[23.] Kiritar, K.R.; Basu, B.D. Indian Medicinal Plants, 2nd ed. Dehradun: International Book Distributors; 2007.
[24.] Frank, H.; Scholl, R.; Aigner, A. Ricin and Ricinus communis in pharmacology and toxicology-from ancient use and "Papyrus Ebers" to modern perspectives and "poisonous plant of the year 2018". Naunyn Schmiedebergs Arch Pharmacol 2018; 392(10): 1181-1208.
[25.] Morya GCK. Ayurvedic approach of eranda (*Ricinus communis* Linn.) on vata vyadhi for green pharmacology. Int J of Theoretical and App Sciences 2016; 8(2); 33-35.
[26.] Trease, G.E.; Evans, W.C. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002 .
[27.] Sofowora, A. Screening plants for bioactive agents. In: Medicinal plants and Traditional Medicines in Africa. Sunshine House, Ibadan, Nigeria: Spectrum Books Limited; 1993. p.134-156.
[28.] Harborne, J.B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. Chapman and Hall, London, New York; 1973. p. 49-188.
[29.] Singleton, V.L.; Orthofer, R.; Lamuela-Ravento, R.M. Analysis of total phenols and other oxidation substrates and oxidants by means of Folin-Ciocalteau reagent. Methods Enzymol 1999; 299;152-178.
[30.] Chang, C.; Yang, M.; Wen, H.; Chern, J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10;178-182.
[31.] Ahmed, F.; Fatima, M.; Saeed, S. Phenolic and flavonoid contents antioxidative potential of epicarp and mesocarp of Lageneria siceraria fruit: a comparative study. Asian Pac J Trop Med 2014; 7; S249-S255.
[32.] Battistelli, M.; De Sanctis, R.; De Bellkis, R.; Cucchiarini, L.; Dacha, M.; Gobbi, P. Rhodiolarosea as antioxidant in red blood cells: Ultrastructural and haemolytic behaviour. Eur J Histochem 2005; 49; 243-254.

[33.] Mizushima, Y.; Kobayashi, M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968; 20(3); 169-173.
[34.] Chandra, S.; Chatterjee, P.; Dey, P.; Bhattacharya, S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Med 2012; 2(1); 178-180.
[35.] Oyedepo, O.O.; Femurewa, A.J. Anti-proteinase and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpiodes and Tetrapleura tetraptera. Inter J Pharmacogn 1995; 33;65-69.
[36.] Sakat, S.; Juvekar, A.R.; Gabhire, M.N. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharma and Pharmacological Sciences 2010; 2(1); 146-155.
[37.] Sadique, J.; Al-Rqobahs, W.A.; Bulghaith, E.I.; Gindi, A.R. The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. Fitoterapia 1989; 60;525-532.
[38.] Shinde, U.A; Kulkarni, K.R.; Phadke, A.S.; Nair, A.M.; Mungantiwar, A.A.; Dikshit, V.J.; Saraf, M. N. Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. Wood Oil. Indian J Exp Biol 1999; 37(3); 258-261.
[39.] Sutar, J.; Monalisa, K.; Pati, K.; Chauhan, V.B.S.; Behera, S. Qualitative and quantitative phytochemical analysis and antioxidant activity of *Curcuma amada* Roxb: An important medicinal plant. Plant Archives 2020; 20(2):193-196.
[40.] Ribeiro PR, de Castro RD, Fernandez LG.Chemical constituents of the oilseed crop *Ricinus communis* and their pharmacological activities: A review. Ind.Crops Prod 2016; 91; 358-376.
[41.] Mohammed, N.I.; Albozachari, J.M.K. Use of Iraqi castor (Ricinus communis) leaf extract as anti-inflammatory in treatment of skin wounds in rabbits. Medico-legal Update 2020; 20(3); 1321-1325.
[42.] Sharma, P.; Toppo, F.A.; Sudheesh, M.S.; Rajak, H.; Pawar, R.S. *Curcuma amada* roxb. (Rhizomes) promotes wound healing process. Curr Tradit Med 2015; 1(3); 217-223.
[43.] Ferraz, C.R.; Carvalho, T.T.; Manchope, M.F.; Artero, N.A.; Rasquel-Oliveria, F.S.; Fattori, V.; Casagrande, R.; Verri, W.A.Jr. Therapeutic potential of flavonoids in pain and inflammation mechanisms of action, pre-clinical and clinical data and pharmaceutical development. Molecules 2020; 25(3); 762.
[44.] Hait, M.; Deepak J. Physicochemical and phytochemical exploration on the non- aerial part of *Curcuma amada*. J Pharmacogn and Phytochem 2018:7(6); 1306-1309.
[45.] Saini, A.K.; Goyal, R.; Gauttam, V.K.; Kalia, A.N. Evaluation of anti-inflammatory potential of *Ricinus communis* Linn leaves extracts and its flavonoids content in wistar rats. J Chem. Pharm. Res 2010; 2(5); 690-695.