



Invitro Antioxidant and Anti-inflammatory Studies of Hadjod Plant: An Important Traditional Indian Medicinal Plant

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ABSTRACT

Cissus quadrangularis (Hadjod) is an indigenous medicinal plant of India. It is well documented in Ayurveda as a general tonic and analgesic with bone fracture healing properties. The present investigation deals with the study of invitro antioxidant and anti-inflammatory potential of ethanolic and methanolic stem extracts of this plant. The stem 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 hadjod extract was examined by protease inhibition activity, albumin denaturation inhibition assay and membrane stabilization. TLC profiling of both ethanolic and methanolic extracts of Hadjod plant showed presence of phytochemicals with R_f values 0.85, 0.80, 0.67 and 0.75 respectively. The highest total phenolic (600±0.153ug GAE/gm extract) and flavonoid (2.5±0.421mg QE/gm extract) content were observed with ethanolic extract of *C. quadrangularis* compared to methanolic extract. Results demonstrated that the ethanolic extract showed significant anti-inflammatory activity by inhibiting heat induced albumin denaturation (90.9±0.65% antidenaturation activity), haemolysis (with 30.7±0.34% haemolysis inhibition) and antiprotease activity. The results indicate that the ethanolic stem extract of Hadjod plant can be potential source of anti-inflammatory agent. Our future work will focus on the identification and validation of bioactive anti-inflammatory agents for their use in modern medicine.

Keywords: *Cissus quadrangularis*, antioxidant, anti-inflammatory, flavonoids, TLC, haemolysis

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicine, nutraceuticals, food supplements, pharmaceutical and chemical entities for the synthetic drugs. India is a country rich in indigenous herbal resources with almost 20,000 plant species of which about 2500 have medicinal value[1]. The medicinal value of these plants lie in the phytochemicals such as flavonoids, polyphenols, alkaloids, tannins, glycosides and saponins which are deposited in their specific parts such as leaves, bark, stem, seeds, root, fruits etc[2]. Phytochemicals are known to possess antioxidant, antimicrobial and anti-inflammatory activities due to which they have got countless medicinal value[3]. They play a vital role against many inflammatory diseases such as cancer, diabetes, arthritis etc without any side effects and thus are considered as “friendly medicines” [4]. *Cissus quadrangularis* is one such plant which is been studied for its medicinal properties mainly for bone related complications, obesity, gastrointestinal disorders [5].

Cissus quadrangularis is a medicinal herb of the Vitaceae family and commonly known as Veldt Grape or Devil's Backbone. It is known as Hadjod in Hindi and Asthisamharaka in Sanskrit [6]. The whole plant including stem, leaves and roots possess medicinal properties. It acts as a drug for local tissue injury and provides relief from wound problems and swelling issues due to inflammation [7].

Free radicals are highly reactive molecules that create oxidative stress in our body and cause many diseases by causing local inflammation. Inflammation results in pain, redness and swelling at the affected site which can be managed by anti-inflammatory agents[8]. There have been some preliminary reports about the invitro antioxidant properties of its stem[9]. Available data on invitro anti-inflammatory activity of stem of this plant remains meagre. The possibility of anti-inflammatory activity of Hadjod plant may be due to presence of phytochemicals in stem. The present study involves determination of antioxidant and mainly anti-inflammatory activity of alcoholic extract of stem by invitro assays which has not been reported till date.

MATERIALS AND METHODS

Collection of Plant Materials: The plant of *Cissus quadrangularis* (Figure1)was collected from a medicinalplant nursery and was grown in terrace garden.

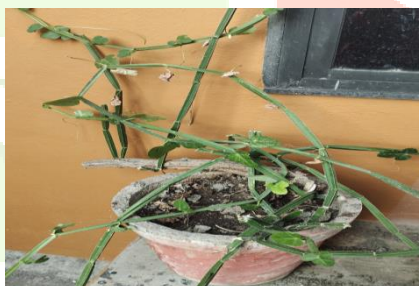


Figure 1: *Cissus quadrangularis* plant

Plant Extract Preparation:The stems (Figure 2)of Hadjod plant were peeled, chopped and grinded in suitable solvent with the help of mortar and pestle. The extracts of the stem wereprepared by soaking 20 gms of finely ground stemin 80 ml of 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.



Figure 2: Stems of *C quadrangularis*

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.

Qualitative phytochemical screening

The different qualitative chemical tests were performed to detect various phytochemicals present in stem of hadjod plant [10, 11, 12]. The tests were performed as follows (Table1):

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.

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.

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.

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_2SO_4 . Appearance of reddish-brown colour at junction of two liquid and bluish green colour of acetic acid layer indicates the presence of glycosides.

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.

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.

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.

Table 1: Qualitative analysis of phytochemicals

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 ₂ SO 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 ₂ SO 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 ₂ SO ₄ along the sides of test tube	Chloroform layer appears red and acid layer shows greenish yellow colour.

Total Phenolic Content: Total phenol content was determined using the Folin- Ciocalteu reagent[13]. 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.

Total Flavonoid Content: The standard Aluminium Chloride method was used to determine the total flavonoid content (TFC) in stem extract of Hadjod plant [14]. An aliquot of 0.1 ml of test sample was mixed with 1 ml 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 from 100 µg/ml to 500 µg/ml against blank. The concentration of flavonoid in the test samples was calculated from the calibration plot.

Ferric Reducing Power Assay (FRPA): Ferric ion reducing power was measured by colorimetric method[15]. Reducing power is a reflection antioxidant activity of any compound. 0.1 ml of stem extracts were mixed with 1 ml sodium phosphate buffer (0.2 M; pH=6.6) and 1 ml 1% potassium ferricyanide and incubated at 50 °C for 20 minutes. After that, 1 ml of 10% TCA was added to the mixture and centrifuged at 3000 r.p.m. for 10 minutes. 1.5 ml supernatant was then added to 1.5 ml of distilled water and 0.1 ml of 0.1% ferric chloride (FeCl₃). The mixture was left aside at room temperature for 10 minutes and then O.D. was recorded at 700 nm using spectrophotometer. Ascorbic acid (1 mg/ml) was used as standard in concentrations of 25-125 µg/ml. Higher reducing power is indicated by high absorbance. All assays were run in triplicates.

Total Antioxidant Capacity (Phosphomolybdate Assay): 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[16]. 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 phosphomolybdate 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 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 µg/ml. The antioxidant capacity was reported as mg of ascorbic acid equivalents (AAE) per gram of extract.

Assessment of invitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of Hadjod plant was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al [17] and Chandra et al [18] followed with minor modifications. The reaction mixture consisted of test extracts (0.2 and 0.4 ml), 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 °C for 5 min and then heated to 70 °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 = $(\text{Abs Control} - \text{Abs Sample}) \times 100 / \text{Abs control}$

Antiproteinase action

The test was performed according to the modified method of Oyedepo et al [19] with some modifications. The reaction mixture consisted of 100 µl trypsin (10 µg/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% CuSO₄ 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.

Membrane stabilization

The study was performed according to Sakat et al. [20], Sadique et al. [21] and Shinde et al. [22] with minor modifications as follows:

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 10 min 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.

Heat induced haemolysis

The reaction mixture consisted of 20 µl test extract, 100 µl of 10% RBCs suspension and 3.0 ml 10 mM PBS, pH 7.4. 20 µl 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 °C for 30 min. 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 = $(\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$

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 $p < 0.05$.

RESULTS AND DISCUSSION

Percentage Yield and Color of Plant Extract

The percentage yield and color (Figure 3) of each extract is presented in Table 2.

Table 2: Percentage Yield and Color of Stem Extract

Plant Extract	Percentage Yield	Color
Ethanol	90%	Dark Olive Green
Methanolic	85%	Olive Green



Figure 3: Methanolic and ethanolic crude extract of *C. quadrangularis*

Thin Layer Chromatographic Studies

TLC profiling of ethanolic and methanolic stem extracts 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 4 and comparative analysis is presented in Table 3. The different R_f values of ethanolic and methanolic stem extract indicated that phytochemicals extracted in both the extract are alike and are important in antioxidant activity of this plant.

Table 3: TLC of ethanolic and methanolic extract of *C. quadrangularis*

S.No.	Fraction	No. of spots	Colour	R_f
1	Ethanol	2	Green	0.85
			Yellow	0.80
2	Methanol	2	Green	0.81
			Yellow	0.75

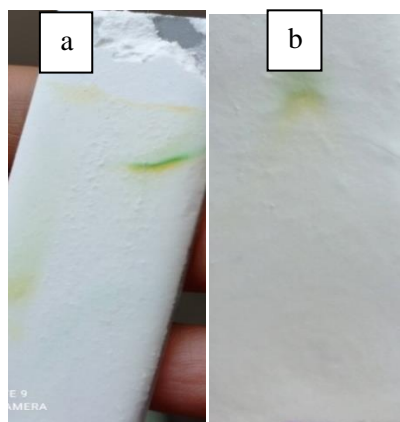


Figure 4: TLC profile of (a) ethanolic and methanolic (b) stem extract of Hadjod plant

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 and methanolic stem extracts of Hadjod demonstrated presence of various secondary phytochemicals like flavonoids, terpenoids, glycosides, steroids (Table 4). The colored reactions for each chemical test are presented in Figure 5.

Table 3: Qualitative phytochemical analysis of stem of Hadjod plant

S.No.	Cissus quadrangularis	Ethanolic Stem Extract	Methanolic Stem extract
1.	Flavonoids	++	+
2.	Terpenoids	++	+
3.	Glycosides	+	+
4.	Saponins	-	-
5.	Tannins	-	-
6.	Phenols	-	-
7.	Steroids	++	+

Present + Strongly present ++ Absent -

Phytochemicals like flavonoids, terpenoids, glycosides and steroids are present in both ethanolic and methanolic stem extracts of Hadjod. Saponin was absent in both the ethanolic and methanolic stem extracts. Polyphenols and tannins were not observed in extracts when tested with FeCl₃, may be they are conjugated or involved in complex formation. Ethanolic stem extract contains good amount of flavonoids and terpenoids as compared to methanolic stem extract. These phytochemicals were better extracted in ethanol than methanol which may contribute to its high antioxidant activity. These results suggested that phytochemicals like flavonoids, terpenoids, steroids are the active constituents in stem of Hadjod. The result of this study is agreed with the previous study reported by Teware et al (2011) [23].

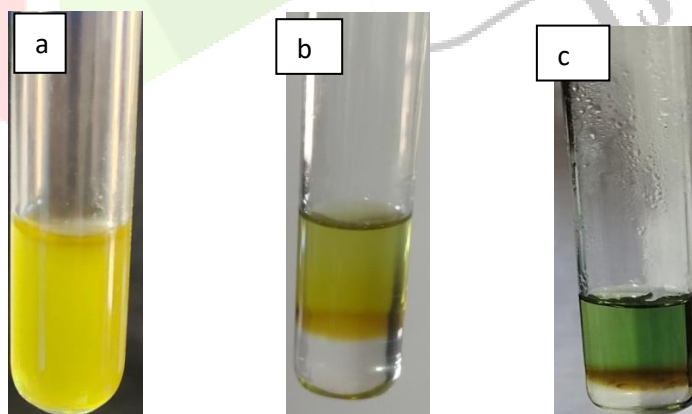


Figure 5: Coloured Reactions for Phytochemical Assays of ethanolic stem extract of Hadjod plant (a) Flavonoids, (b) Glycosides (c) Terpenoids

Total Phenolic Content (TPC)

The total phenolic content of ethanolic and methanolic stem extracts of Hadjod plant was estimated using gallic acid as standard from the calibration curve as shown in Figure 6. They were 600.4 ± 0.153 and 306 ± 0.458 ug gallic acid equivalents (GAE)/gm of sample in ethanolic and methanolic extracts of Hadjod plant. The phenolic content in ethanolic stem extract was almost double than that of methanolic extract. The experimental results obtained from the extracts are presented in Table 4.

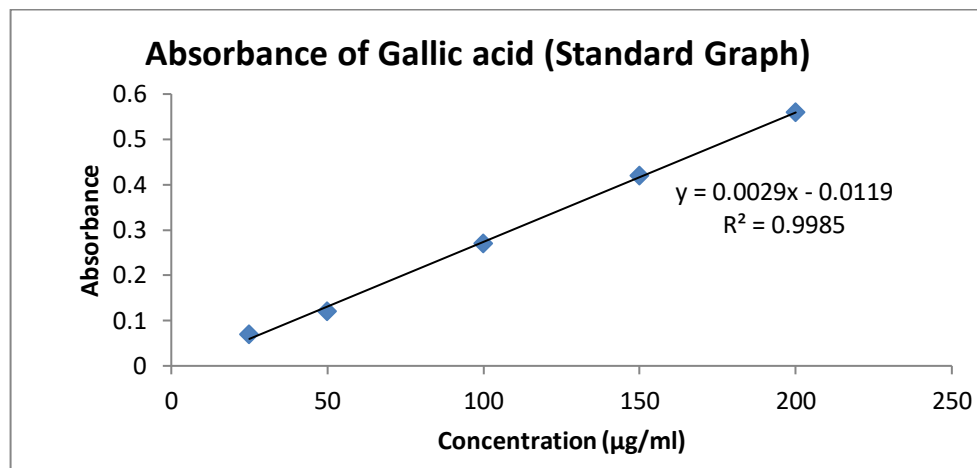


Figure 6: Standard Curve of Gallic Acid

Total Flavonoid Content (TFC)

Total flavonoid contents were extrapolated from the straight line equation of quercetin standard curve (Fig.7). TFC obtained for ethanolic and methanolic stem extracts of Hadjod plant are 2.5 ± 0.421 and 1.6 ± 0.276 mg quercetin equivalent (QE)/g extract respectively (Table 4). Thus, total flavonoid content in ethanolic stem extract is significantly higher than methanolic extract.

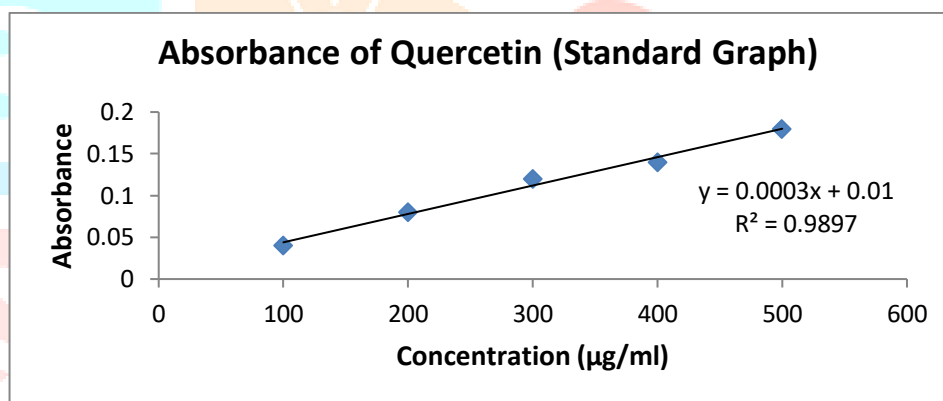


Figure 7: Standard Curve of Quercetin

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 4** The ethanolic stem extract of Hadjod plant revealed maximum antioxidant potential as 262.2 ± 0.361 µg ascorbic acid equivalent (AAE)/gm extract. Lower FRPA value was observed for methanolic extract of Hadjod plant as 112.1 ± 0.400 µg AAE/gm. Ascorbic acid at the concentration of 1 mg/ml was used as the reference antioxidant for comparison.

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 4**. Ethanolic extract of Hadjod plant demonstrated the highest antioxidant potential (330.7 ± 0.757 µg AAE/gm) and the lowest were in methanolic extract (231.3 ± 0.491 µg AAE/gm). Ascorbic acid at the concentration of 1 mg/ml was used as the reference antioxidant for comparison.

Table 4: Quantitative phytochemical and antioxidant assay in Hadjod stem extract

Extract	TPC (ugGAE/gm extract)	TFC (mgQE/gm extract)	FRPA (ugAAE/gm extract)	TAC (ugAAE/gm extract)
Ethanolic	600±0.153	2.5±0.421	262±0.361	330.7±0.357
Methanolic	306±0.458	1.6±0.276	112±0.400	231±0.491

Values are expressed as mean ±SD for three determinations

Anti-inflammatory studies

Inhibition of albumin denaturation

Denaturation of protein is the major cause of inflammation. The ability of ethanolic and methanolic stem extracts of Hadjod plant to inhibit protein denaturation was studied for their anti-inflammatory action. The ethanolic stem extract was significantly effective in inhibiting heat induced albumin denaturation as compared to methanolic extract. Maximum inhibition of 90.9±0.65% was observed for 0.4 ml of ethanolic stem extract (250mg/ml) while for similar volume, methanolic stem extract showed 54.5±0.54% inhibition. Aspirin was used as a standard anti-inflammatory drug and it showed 63±0.75% inhibition for 0.4 ml as compared to control. Results are presented in Table 5.

Table 5: Effect of Hadjod stem extract on albumin denaturation

Sample	Concentration (mg/ml)	Volume (ml)	Albumin denaturation (% inhibition)
Ethanolic	250	0.4	90.9±0.65
Methanolic	250	0.4	54.5±0.54
Aspirin	15	0.4	63±0.75

Values are expressed as mean ±SD for three determinations

Proteinase inhibitory activity

The ethanolic stem extract of Hadjod plant 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 stem extract 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 (Fig.8). Control showed light blue/purple colour due to action of protease on casein. Methanolic stem extract 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.

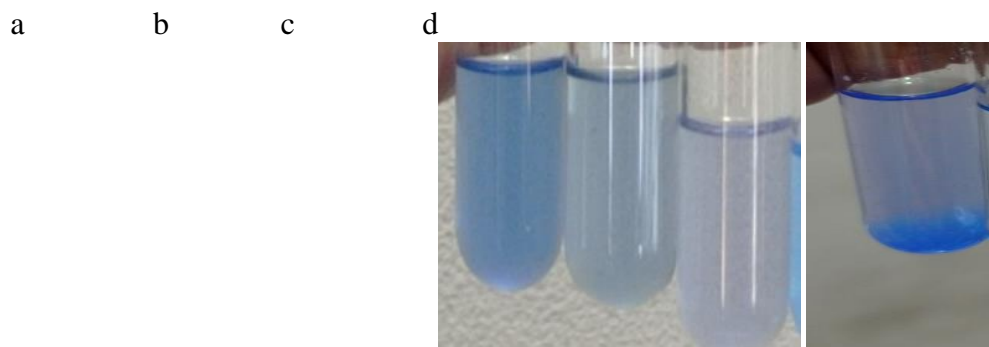


Figure 8: Antiproteinase activity of Hadjod stem extract a) Ethanolic extract; b) Methanolic extract; c) Control; d) Aspirin

Membrane stabilization

Stabilization of RBCs membrane was studied to establish an additional mechanism for anti-inflammatory action of ethanolic and methanolic stem extract of Hadjod 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 stem extract significantly inhibited heat induced hemolysis of RBCs with maximum inhibition $30.7\pm 0.34\%$ at 250mg/ml. Methanolic stem extract showed $23.76\pm 0.56\%$ hemolysis inhibition which was least. Aspirin, standard drug showed inhibition, $28.97\pm 0.67\%$ at 15mg/ml (Table 6). As seen in Figure 9 inhibition of hemolysis is inferred in ethanolic and methanolic extracts by light red colouration as compared to control.

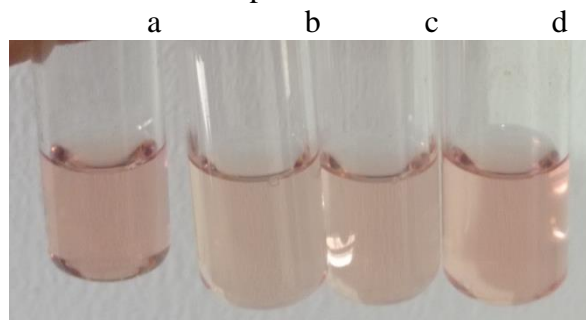


Figure 8: Hemolysis inhibition by Hadjod stem extract a) Control; b) Ethanolic extract; c) Methanolic extract; d) Aspirin

Table 5: Effect of Hadjod stem extract on membrane stabilization

Sample	Concentration (mg/ml)	Volume(ml)	% hemolysis inhibition
Ethanolic	250	0.02	30.7 ± 0.34
Methanolic	250	0.02	23.76 ± 0.56
Aspirin	15	0.02	28.97 ± 0.67

Values are expressed as mean \pm SD for three determinations

DISCUSSION

The results of our invitro studies on the ethanolic stem extract of Hadjod plant demonstrate that it has strong antioxidant and anti-inflammatory property which could be potential source of anti-inflammatory drug. Antioxidant potential is clearly revealed in FRPA and TAC assay. The inhibition of albumin protein denaturation, antiproteinase action and membrane stabilization was studied to establish the mechanism of anti-inflammatory activity of stem of Hadjod plant. The results of these mechanisms showed greater potential of ethanolic stem extract of hadjod plant to be used as anti-inflammatory agent. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activity of many plants [24]. Hence anti-inflammatory activity of stem extract of Hadjod plant 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 anti-arthritic property of *C. quadrangularis* by Vaijayanthimala et al (2018) [25].

CONCLUSION

The results of the study revealed that the stem extract of Hadjod plant possessed significant antioxidant and anti-inflammatory activity in ethanolic fraction than compared to methanolic fraction. The ethanolic stem extract showed better anti-inflammatory activity than standard anti-inflammatory drug aspirin. Hence it can be used in the management of inflammation but further investigations in terms of profiling of phytochemical content on Hadjod plant using HPTLC/HPLC is essential to figure out the active candidate or chemical entity that is mainly responsible for this activity. The identified molecule then can be used in formulation of anti-inflammatory drug for therapeutic applications either internally or externally. During this time of pandemic where infections and resultant inflammation are literally affecting humans adversely, medicinal plants like *C. quadrangularis* can provide better alternative.

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